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CANCER RESEARCH

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Assay of Frozen Mouse Mammary Carcinoma for the Mammary Tumor Milk Agent*

JOHN J. BITTNER, PH.D., AND DAVID T. IMAGAWA, PH.D.

(From the Division of Cancer Biology, Department of Physiology, and Department of Bacteriology and Immunology, University of Minnesota Medical School, Minneapolis 14, Minn.)

In a series of publications during the past year Gye and his associates (28, 29, 33–35) interpreted their data to imply that when various types of cancers from mice were maintained at −79° C., the malignant cells "are readily killed by extreme cold, but the dead malignant cells are nevertheless capable of starting a new, strictly new, tumour in virtue of the intrinsic virus which they contain" (28). It was suggested that the active form of the virus, changed from a latent form because of freezing, was capable of altering the connective tissue of the injected host to sarcoma or the normal mammary gland to mammary carcinoma, depending upon the type of cancer being tested. Normal and frozen embryonic tissue served as controls for the cancer studies (Mann, 32).

Active dried tissue was obtained by Gye (28) from fresh sporadic tumors which had developed spontaneously in mice of the R3 and C3H stocks. However, positive results with dried tumors were secured only when the tissue was minced (14) and dried in a Craigie desiccator (15). When the tissues were subjected to the Knox method of drying, only negative results were obtained (29).

Gye (28) postulated that the negative results obtained by other investigators were due to imperfect technic and stated that their experimental data indicated "that cancer has a continuing cause and that this, in mammals as in birds, is a virus."

Mann (33–35) injected frozen spontaneous and transplanted mammary cancers and explained the development of tumors on the basis that the mammary tumor milk agent-virus was liberated in its active form following freezing, since mammary tumors arose at the site of injection of the suspended frozen tumor-mince. The tumors developed as readily in males as in female mice, but only when the frozen tissue was injected into the mammary gland region, because of what the author called "selective infectivity." She found that, within limits, the longer the tissue was frozen, the more tumors developed, and the interval between injection and the appearance of the tumors was related to their rate of growth. Fast growing cancers produced growths within 10 days, while slow growing ones might be delayed for 7–12 weeks following the inoculation of their frozen suspensions. Mann (35) also demonstrated that mammary tumors could be produced by the injection of tumors which had been frozen and dried *in vacuo* (15), provided no thawing occurred before the material was dry.

Gye, Begg, Mann, and Craigie (29) reported that some tumors could withstand a succession of freezing and thawing, which they claimed completely excluded the persistence of tumor cells, but Mann (34) observed a progessive deterioration of activity of mammary tumors after a second thawing. Individual tumors differed, but in no instance did a tumor inactive after a second thawing produce tumors after a third.

The exact age of the animals at the time of injection was not stated except that "Although all came

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from high cancer lines, they were used before they were old enough to develop sporadic tumours, and in all cases the tumours obtained were at the site of inoculation" (33). The tumor suspension was "inoculated in the right mammary region of mice of the same strain as the tumours" (33).

The studies to be reported here are concerned with the propagation of mammary tumors following the injection of suspensions of frozen mammary carcinoma and the comparative activity of transplanted fresh tissue of the same tumors. Assays were made of the fresh and frozen mammary tumors for the mammary tumor milk agent-virus by the injection of cell-free extracts using different routes of administration. A tumor that resulted after the inoculation of a frozen suspension of tumor-mince was also tested for the agent.

MATERIALS AND METHODS

Mice of several inbred stocks and one group of F_1 hybrids were used in the various experiments. All the animals were susceptible for the development of spontaneous mammary cancer, and all possessed the mammary tumor milk agent except the fostered lines of the Z (C3H) and A strains and their hybrids. These are referred to as the Zb and Ax lines; their reciprocal hybrids as either $ZbAxF_1$ (Zb♀ × Ax♂) or $AxZbF_1$ (Ax♀ × Zb♂).

The spontaneous mammary tumor No. 8415A arose in an AZF_1 breeder produced by mating an A female with a Z male. This particular tumor was transplanted by the trocar method in F_1 hybrids without the milk agent. In eight passages, a total of 65 mice were inoculated, and the grafts grew progressively in all. During the first three passages the tumor was transferred every 4 weeks; after that, because of the increased growth of the transplants, inoculation was made approximately every 2 weeks. Within 6 days some growths attained a size of 0.75 cm. in diameter.

One of the tumors which arose, following the injection of the frozen tumor-mince of AZF_1 No. 8415A, was continued as AZF_1 No. 8415B. This tumor was maintained for three passages in either $AxZbF_1$ or $ZbAxF_1$ hybrids, and none of the 23 mice which were inoculated was resistant to the grafts.

A mammary tumor from a breeding Z (C3H) female was designated as tumor No. 8044 and was transplanted in F_1 hybrids made by reciprocal matings of A and Z mice without the milk agent. This transplant has been continued for 40 passages, and 238 mice have been inoculated. All have shown progressively growing tumors, and the mice usually succumb within 3–4 weeks after they have been inoculated. The tumors may attain the size of 1 cm. in diameter within 1 week.

The frozen suspensions of tumor tissue were prepared according to the technic outlined by Craigie (16). The tumors were removed and minced in a tissue press through a screen with holes 0.0410 inches in diameter (Fig. 1). A screen with openings 0.0175 inches in diameter was substituted for the first screen on the cylinder, and the tissue was forced through the press a second time. Most of the connective tissue had been removed in the former mincing. This press, with the exception of the screen with the smaller openings, has been in use for at least 10 years. Equal volume of 5.3 per cent dextrose was added to the tumor-mince, and the

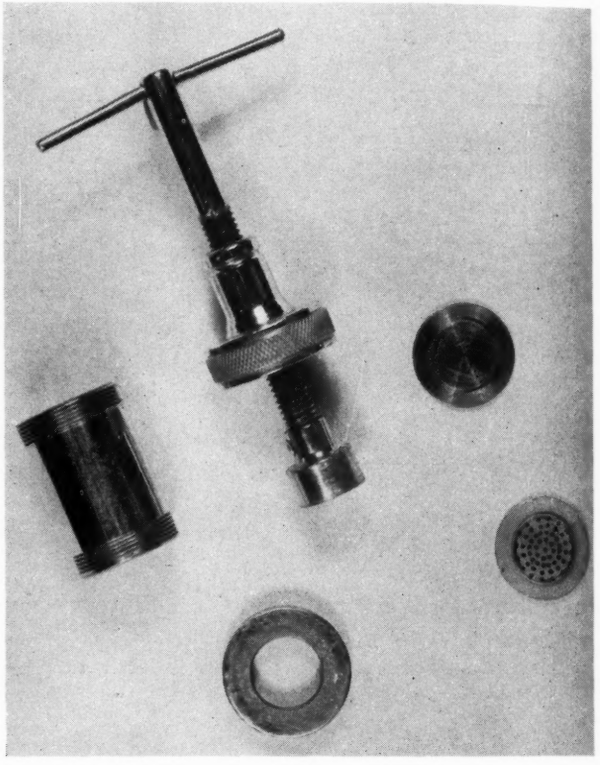

FIG. 1.—Tissue press used for the preparation of the tumor-mice. The cylinder of the press may be made to any desired length; the one illustrated is 1.75 inches in length, outside diameter, 1.13 inches, and the diameter of the tube, 0.75 inches. It is made of chromium-plated brass; another in use was made of stainless steel and has a cylinder of 2.75 inches (approximate capacity, 10 gm.). The pressure screw, with plunger attached, operates through the top plate of the press which screws onto the cylinder. The plunger is 0.749 inches, which gives a minimum clearance in the tube. The outer edge of the screen is 0.7 inches, and a "hump" of 0.04 in the center fits tightly into the tube of the cylinder. The openings in one screen are 0.0410, in the other 0.0175 inches. The base plate holds the screen in place.

The tissue may be inserted from either the top or the bottom of the cylinder by the removal of the respective end plate from the cylinder.

suspension was stored in glass ampoules, 2 cc. per vial, which were sealed. The ampoules were frozen in cellosolve and dry ice and stored in a thermos jug with dry ice at a constant temperature of −79° C.

For testing the ability of the frozen tumor-mince to produce tumors, the ampoules were transferred to a water bath kept at 37° C. until the tissue had thawed. Six cc. of saline was added to the contents of each tube, and the suspension was injected so that each animal received an amount equal to 0.05 gm. of the original tumor-mince. The site of injection is given for each experiment.

In addition to testing the frozen tumor-mince, cell-free extracts were also employed. Details will be given for the various studies. Other observations were made on assays of nonfrozen transplants of the same tumors for the milk agent.

The thawed mince of tumor No. 8415A from four vials was pooled, 24 cc. of saline was added, and the suspension was centrifuged for 20 minutes at approximately 2,500 r.p.m. The supernatant was removed and recentrifuged for the same time. The final supernatant was injected subcutaneously near the left axillary region of the mice listed in Table 1 and referred to above. All animals, regardless of stock, failed to show tumors at the site of injection during the period they were under observation (1/6/50–2/23/50).

On February 23 (48 days after the injection of either the thawed tumor-mince or the centrifugate supernatant), some of the mice in which no tumors developed (Z, Ax, and F_1) were inoculated simultaneously with grafts of tumor No. 8415A (right) and tumor No. 8415B (left). Both tumors grew progressively in the $ZbAxF_1$ mice that had been resistant to the previous injections, and the animals were killed on 3/15/50 with large transplanted tumors. Mice of the A and Z stocks were all resistant to the grafts from the AZF_1 tumors (Table 1).

TABLE 1

DEVELOPMENT OF MAMMARY TUMORS AT THE SITE OF INJECTION OF THE SUSPENSION OF FROZEN TUMOR-MINCE OF AZF_1 TUMOR NO 8415A*

Stock	Age when injected	No. inoc.	Sex	Inoc. frozen tumor, 1/6/50: Tumor susp. AZF_1 8415A tumors	Inoc. frozen tumor, 1/6/50: Supernatant tumor susp. AZF_1 8415A tumors	No. inoc.	Inoculation of fresh tumor grafts 2/23/50: 8415A tumors	Inoculation of fresh tumor grafts 2/23/50: 8415B tumors
Z (C3H)	3 mo.	7	♀	0	0	7	0	0
Z (C3H)	"	2	♂	0	0	0		
Zb	18 mo.	4	♀	0	0	0		
A	2 mo.	2	♀	0	0	0		
A	"	6	♂	0	0	0		
Ax	1 mo.	7	♀	0	0	7	0	0
Ax	"	6	♂	0	0	0		
$ZbAxF_1$	5 wk.	7	♀	3†	0	4	4	4
$ZbAxF_1$	"	6	♂	2‡	0	4	4	4

* Frozen 1/4/50, injected 1/6/50.

† Tumors which developed were assayed for the milk agent 2/9/50 (Table 4).

‡ One tumor was inoculated as AZF_1 8415B. Some mice which were negative to the injection of the frozen material were inoculated on 2/23/50 with fresh tissue grafts of tumors 8415A and 8415B.

EXPERIMENTAL RESULTS

Following the first passage of the AZF_1 tumor No. 8415A in F_1 hybrids without the milk agent, the transplants from several animals were frozen on 1/4/50 and maintained at −79° C. for 48 hours. Following thawing, each mouse was injected with 0.05 gm. of the original tumor tissue subcutaneously in the right axillary region. With the exception of four Zb females which were 18 months of age, all the test animals were from 5 weeks to 3 months of age at the time of injection.

All mice, twenty females and fourteen males, of the A and Z (C3H) stocks failed to develop tumors, but tumors appeared in three of the seven females and two of six males of the $ZbAxF_1$ hybrids (Table 1). One of the tumors from a male was continued as No. 8415B.

The AZF_1 tumor No. 8415A was inoculated on 12/7/49 in F_1 hybrids (mice without the milk agent). The first passage transplants were tested on 1/6/50 for the mammary tumor milk agent by our usual technic (see 11). The tumors were minced with the tissue press, ground with sand, and extracted with distilled water (1:10 by weight). The suspension was centrifuged for 10 minutes, and the supernatant was recentrifuged for the same period (clinical centrifuge). The second supernatant was further diluted, based upon the weight of the wet pressed tissue, and injected either subcutaneously or intraperitoneally into 21–

25-day-old ZBC females. These are susceptible animals which do not possess the milk agent; the incidence of tumors in controls is less than 2 per cent (see 11). No mammary tumors have developed in any of the injected mice after 185 days (Table 2).

The frozen tumor-mince of the AZF_1 tumor No. 8415A, frozen on 1/4/50, was assayed on 1/11/50

TABLE 2

ASSAY OF THE FIRST PASSAGE TRANSPLANTS (FRESH TISSUE) OF THE AZF_1 TUMOR NO. 8415A FOR THE MAMMARY TUMOR MILK AGENT*

GM. EQUIV. INJECTED	SUBCUTANEOUS INJECTION		INTRAPERITONEAL INJECTION	
	No.	Tumors	No.	Tumors
2×10^{-2}	33	0	22	0
10^{-3}	35	0	45	0

* Data tabulated after 185 days.

TABLE 3

ASSAY OF THE FROZEN FIRST PASSAGE TRANSPLANTS OF THE AZF_1 TUMOR NO. 8415A FOR THE MAMMARY TUMOR MILK AGENT*

GM. EQUIV. INJECTED	SUBCUTANEOUS INJECTION		INTRAPERITONEAL INJECTION	
	No.	Tumors	No.	Tumors
2×10^{-2}	33	0	35	2
10^{-3}	33	0	34	0

* Tissue frozen 1/4/50; tested 1/11/50. Data tabulated after 180 days.

TABLE 4

ASSAY OF TUMOR FOR THE MILK AGENT

GM. EQUIV. INJECTED	SUBCUTANEOUS INJECTION		INTRAPERITONEAL INJECTION	
	No.	Tumors	No.	Tumors
Section A				
2×10^{-2}	33	0	28	0
10^{-3}	28	0	30	2
Section B				
2×10^{-2}	16	0	53	0
10^{-3}	50	0	32	0

Section A: Assay of tumor for the milk agent which developed following the injection of the frozen tissue of the AZF_1 tumor 8415A. Frozen tissue injected 1/6/50, tumors tested 2/9/50. The data were tabulated 161 days later, first tumor after 138 days.

Section B: Assay for the agent in the third passage transplants of tumor 8514B. Data recorded after 76 days.

for the so-called "active" (Mann, 32) milk agent. The second supernatant was injected subcutaneously into 66 and intraperitoneally into 69 young ZBC females (Table 3). To date, after 180 days, two tumors have developed in the animals following the intraperitoneal administration of the extract.

Two tumors which developed following the subcutaneous injection on 1/6/50 of the frozen tumor-mince of the AZF_1 tumor No. 8415A were tested on 2/9/50 for the milk agent by using the fresh tissue. Two fractions of the second supernatant were used by different routes of administration. One animal that received the material intraperitoneally developed a tumor after 138 days (Table 4, Section A), and another in the same series had a mammary tumor at a later age.

The AZF_1 tumor 8415B, derived from the frozen tissue of No. 8415A, was carried for three passages and assayed for the milk agent (Table 4, Section B). All the test animals have remained free of tumors for 66 days.

The Z (C3H) tumor No. 8044 has been found to possess the mammary tumor milk agent after it has been transplanted for 30 passages in either $ZbAxF_1$ or $AxZbF_1$ hybrids—mice which themselves did not carry the agent. These data are tabulated in Table 5. Only preliminary observations may be given for many of the groups. The earliest tumor appeared in a test animal (ZBC) which was 154 days of age, and the average cancer ages for completed groups ranged from 311 to 364 days of age.

Transplants of the 33d passage of the Z tumor No. 8044 were minced and frozen in dextrose on 2/3/50. The tissue was maintained at −79° C. until 2/20/50, when the contents of several ampoules were tested by injecting mice subcutaneously with 0.05 gm. of the original material.

Some animals of the Z and Zb lines and the $AxZbF_1$ hybrids developed tumors at the site of injection, while all mice of the A and D stocks remained free of tumor (Table 6).

Other vials of tumor No. 8044, frozen on 2/3/50, were used on 3/31/50. In this study, mice of the $ZbAxF_1$ generation, when approximately 8 weeks of age, received both subcutaneous and intraperitoneal injections of 0.05 gm. of the original tumor-mince. As seen from the data (Table 7), there was no significant variation in the number of tumors to be observed at the two sites of inoculation. One male with a large subcutaneous tumor could not undergo autopsy to determine if it might also have an internal tumor.

Weights were taken of twelve of the subcutaneous and all the intraperitoneal tumors. Although the subcutaneous tumors were much more hemorrhagic than the abdominal growths, the fluid was extruded before the tumors were weighed. The tumors from the subcutaneous area varied between 0.9 and 3.6 gm.—average, 2.0 gm.; the range for the internal tumors was 0.15–3.3 gm.—average, 1.5 gm. Two animals had several internal masses.

DISCUSSION

At least three causative factors have been found to play a role in the genesis of spontaneous mam-

mary cancer in mice (5; see 8, 37, 9–10 for literature). One of these, the mammary tumor milk agent (4), has the characteristics of an infectious agent or virus and will remain active following filtration (7, 1), lyophilization (6), and desiccation (8, 19–23) even after storage for as long as 2 years (Dmochowski, as reported by Gye [26]). When administered subcutaneously in cell-free extracts, mammary tumors developing at the site of injection are no more common than in other regions of the mammary system, and the time of appearance has not been accelerated when compared with intraperitoneal administration of the material. In general, mice of strains with a high incidence of spontaneous mammary tumors show an earlier average cancer age than do mice in the injection experiments.

Our interest in the problem of freezing mammary cancer was stimulated by the reports of Mann (33–35) that an "active" milk agent virus might be liberated from the frozen mammary tumor cells which would be capable of producing mammary cancer in a matter of days, instead of months, following its injection.

To test the ability of a frozen suspension of a mammary tumor-mince to produce cancer following subcutaneous injection, we selected for the first series of studies a spontaneous mammary carcinoma which had developed in a hybrid female derived by crossing a female of the cancerous A stock with a male of the cancerous Z or C3H strain. This was tumor No. 8415A from an AZF_1 female. The tumor was transplanted into F_1 hybrids which do not possess the milk agent. Tumors of this trans-

TABLE 5

ASSAY OF THE SPONTANEOUS Z (C3H) MAMMARY TUMOR NO. 8044 AND TRANSPLANTS OF THE TUMOR, CARRIED IN MICE WITHOUT THE MILK AGENT, FOR THE MAMMARY TUMOR MILK AGENT

Tumor passage	Date of injection	Gm. equiv. injected	Age 1st tumor	No.	Per cent	Av. age in days: Cancer	Av. age in days: Non-ca.
Spontaneous tumor	1/17/48	10^{-2}	168	37	76	328	468
		10^{-4}	154	33	70	311	528
1st	2/17/48 and 3/8/48	10^{-2}	154	90	69	320	352
		10^{-4}	194	71	75	354	443
		10^{-5}	254	47	43	364	485
7th	7/22/49	2×10^{-2}	248	38	79	352	416
		10^{-3}	256	48	56	363	493
18th	4/13/49	10^{-2}	287	24	25*		
		10^{-4}	255	22	9*		
		10^{-5}	318	22	5*		
20th	5/26/49	10^{-2}	225	35	60*		
		10^{-4}	185	39	13*		
		10^{-5}	166	38	8*		
		10^{-6}	245	35	3*		
24th	8/4/49	10^{-1}	221	25	52*		
		10^{-2}	187	62	45*		
		10^{-3}	204	54	24*		
		10^{-4}	181	32	13*		
		10^{-5}	210	29	10*		
30th	12/1/49	10^{-2}	167	50	10*		
		10^{-4}	172	56	7*		
		10^{-5}		60	3*		
		10^{-6}		55	0*		

* Preliminary data, tabulated 221 days after injection of last series.

TABLE 6

TUMORS FOLLOWING THE SUBCUTANEOUS INJECTION OF THE FROZEN TUMOR-MINCE OF Z (C3H) TUMOR, NO. 8044, 33D PASSAGE*

Stock	No.	Sex	Age when injected (weeks)	Tumors
Z (C3H)	6	♀	5–6	5
	5	♂	5–6	3
Zb	6	♀	5	3
	6	♂	5	0
$AxZbF_1$	5	♀	5–6	1
D	7	♀	6	0
	5	♂	6	0
A	6	♀	6	0
	2	♂	6	0

* Tumor frozen 2/3/50, inoculated 2/20/50.

TABLE 7

THE DEVELOPMENT OF TUMORS FOLLOWING THE INJECTION OF THE FROZEN TUMOR-MINCE OF Z TUMOR, NO. 8044*

Stock	No.	Sex	Subcutaneous Tumors	Subcutaneous Av. wt. (gm.)	Intraperitoneal Tumors	Intraperitoneal Av. wt. (gm.)
$ZbAxF_1$	18	♂	14	2.0	11	1.5
$ZbAxF_1$	3	♀	0		2	2.5
$ZbAxF_1$	21	Total	14	2.0	13	1.7

* Each animal received injections in subcutaneous and intraperitoneal sites. The mice were 8 weeks of age when they were inoculated.

plant generation were frozen to −79° C. with the technic outlined above (16).

When a suspension of the frozen AZF_1 tumor was injected subcutaneously into males and females of the A and Z stocks and their F_1 hybrids, tumors appeared at the site of injection in some animals of the F_1 generation, but all mice of the A and Z parental strains remained free of tumor. One tumor which arose in a male F_1 hybrid was transplanted as tumor No. 8415B. A cell-free extract of the frozen tissue failed to produce tumors in any mouse.

Many of the animals which had been resistant to the subcutaneous injection of the frozen material of tumor No. 8415A were reinoculated simultaneously with grafts of fresh tissue of tumors Nos. 8415A and 8415B. All the F_1 mice which had shown a negative reaction to the previous injection of the frozen tissue showed progressive growth of the grafts of both tumors and were dead, with large tumors, within a few weeks. Again, mice of the A and Z stocks were resistant.

In selecting a mammary tumor which had developed in a hybrid mouse, we knew from previous studies (2, 3) that such a tumor would grow progressively, when grafts of fresh tissue were transplanted subcutaneously, in mice of the F_1 generation, whereas few, if any, of the animals of the parental strains would be susceptible to the transplants. The earlier experiments (2, 3) showed that the genes for susceptibility to a transplantable mammary tumor from a hybrid, as the AZF_1, would be transmitted by each parent of the A and Z strains. The mice of the parental strains would all be resistant to grafts of the F_1 tumor, since the mice of one strain would not possess the genes necessary for susceptibility that were transferred by the parent of the other stock to the F_1 progeny.

The results following the subcutaneous injection of the frozen suspension of the AZF_1 tumor were similar to the observations reported by Mann (33) for "mice of the same strain as the tumours," but mice of the parental strains were all resistant. All mice of the F_1 generation and the A and Z inbred strains remained free of tumors when a cell-free extract was injected instead of a suspension of tumor-mince. This demonstrated the importance of the suspended tumor-mince in the development of these tumors.

One tumor which arose following the injection of the frozen mince was continued by transplantation. Grafts of this tumor produced transplanted tumors in animals of the F_1 generation but not in A and Z animals; thus, its characteristics were similar to the original tumor from which it arose. Both tumors grew when transplanted into mice of the F_1 generation which were negative following the injection of the frozen material.

In addition to corroborating the studies of Mann, our data also supported the genetic theory of transplantation advanced by Little and Strong (31), in 1924, and suggested the probability that some tumor cells had survived the low temperature to which they had been subjected. That is, only animals of the same genetic constitution as the mouse which gave rise to the original spontaneous mammary tumor developed mammary tumors following the injection of a frozen tumor-mince; all mice, regardless of their genetic make-up, remained free of tumors when a cell-free extract was tested, although this extract would be expected to possess the mammary tumor milk agent (see 11). As with the usual transplantable mammary tumors, tumors appeared in some mice of the F_1 generation which were free of the milk agent (10), but the presence or absence of the agent in the A and Z mice was of no significance.

Histologic examination of the frozen tumor-mince was not made, since it was doubtful if such observations would have contributed anything to what Craigie (17) concluded when he stated: "I, for one, when examining unfixed and unstained tumour cells under the phase microscope am often uncertain whether they are dead or alive." In a recent presentation of their data, Blumenthal, Walsh, and Greiff (12, 13) had made preparations of their frozen tissues, both normal and cancerous, and concluded that some cells appeared to have survived.

The mammary tumor milk agent may be transferred by the injection of cell-free extracts, as mentioned above. Although the agent from one strain of cancerous animals may have a different activity in mice of another genetic susceptibility for spontaneous mammary cancer, no data have been published which would suggest that the agent from any cancerous strain would not produce some tumors, with the other causative factors also active, in other strains. This fact has been acknowledged by Gye (25), who, in discussing the work of Dmochowski on the recovery of the milk agent from dried cancer tissue, wrote: "Thus it can be concluded that the milk factor derived from any high cancer strain of mice can induce breast cancer in susceptible strains of mice, although their genetic constitution differs from that of the strain from which the milk factor originated." Thus, if freezing mammary tumors liberates the "active" milk agent, this agent should produce mammary tumors within a few days at the site of injection (Mann, 33) in mice of strains other than that in which the tumor had developed.

Every mouse inoculated in our studies was susceptible to the development of spontaneous mammary cancer. In the case of the AZF_1 tumor, it would have the milk agent from the female of the A stock. In previous studies it was determined that when females of the Z (C3H) strain were nursed by females of the A stock, so that they obtained the A stock milk agent, the incidence of mammary tumors was higher in the progeny of these fostered mice when they were maintained as nonbreeders than when they possessed the Z milk agent (11). However, the injection into the mammary gland region of either a suspension of the frozen tumor-mince or the cell-free supernatant of the frozen suspension of the AZF_1 mammary tumor did not induce tumors in mice of the Z stock.

One tumor which developed in a male F_1 hybrid without the milk agent, following the inoculation of the frozen AZF_1 tumor, was also tested by using cell-free extracts and was found to be no more active than either extracts of the frozen material or fresh tumor tissue in animals of the A and Z strains. Several hundred test animals have been injected with these extracts, either subcutaneously or intraperitoneally, and the results to date indicate that this material was no more active than the tissue containing the "latent" (to use Mann's terminology) mammary tumor milk agent. The final results will not be known for many months and may give some interesting data on the comparable activity of the agent in fresh versus frozen tissue as well as different routes of administration.

After 30 passages in mice free of the agent, the agent was found to be present in cell-free centrifugates of the Z (C3H) tumor No. 8044. Transplants of the 33d transfer generation were frozen, and the results following the subcutaneous injection of the frozen tumor suspension were similar to those seen for the AZF_1 tumor, in that tumors arose at the site of injection only in animals of the Z stock and their F_1 hybrids. Again, the genetic relationship between the mice inoculated and the constitution of the tumor inoculated influenced the results—and not an "active virus" liberated from the cancer cells at low temperatures, for mice of other strains were free of tumor after treatment.

As stated above, the mammary tumor milk agent may be demonstrated in filtrates and cell-free extracts (see 8, 9–10, 37), and it seems reasonable to suppose that similar extracts of frozen tissue would likewise contain the virus. Gye and his co-workers (29) centrifuged an extract of a frozen suspension of an induced sarcoma for 15 minutes at 10,000 r.p.m. The sediment was washed with saline and emulsified in 3 cc. of saline. This was injected into six mice, and all developed typical sarcomas. Gye reasoned that "Experiments such as this do not appear to lend any support to the belief that the tumors started by frozen tissues are transplants, i.e., due to division of surviving tumor cells." If viable tumor cells had been present, they would be expected to be found in the resuspended sediment but not in the supernatant; however, the supernatant should have contained any active virus. The latter was not tested.

Mann (33) discussed in detail what she termed the selective infectivity of the so-called active milk virus from frozen mammary tumors for mammary tissue. So that no personal element will enter into the interpretation of her results and the basis for her hypothesis, it seems appropriate to give quotations from her publications. This will be followed for others, as well.

Mann (33) stated that "If inbred strains of mice are used tumour tissue can be successfully grafted in both subcutaneous and intraperitoneal sites. The new tumours are simple clumps of cells of the original mouse growing in the tissues of the new host. If, however, the active form of the Bittner virus has been obtained from living mouse cells it seems highly probable that only cells of mammary tissue can be infected, and that connective-tissue cells cannot respond to it. Since it is well known that the mammary tissue of the mouse is very widespread, and that sporadic tumours can arise almost anywhere on the surface of the body, subcutaneous inoculation of active virus can never offer evidence for or against selective infectivity.

"On the other hand, sporadic mammary cancer never arises inside the peritoneum, and yet successful intraperitoneal grafts of living tumour tissue can be easily obtained. If, therefore, frozen tumour tissue, which as we have seen readily produces subcutaneous mammary cancers, were inoculated intraperitoneally it should produce tumours if it contained living cells, and should not do so if it contained only the active virus. In the experiments . . . qualitative difference in results exists between subcutaneous and intraperitoneal inoculation of refrigerated mammary tumour. The number of failures is too high to be accounted for by any explanation other than the absence of mammary tissue in the site chosen.

"The technique of intraperitoneal inoculation is difficult because of the ease with which a few cells from the surface may be carried into the deeper tissues by the needle and also because of the difficulty of avoiding contamination of the abdominal wall and subcutaneous tissues by the material in the needle during its withdrawal. . . . Again, microscopic examination of subcutaneous and intraperitoneal tumour grafts often reveals the presence

of hair follicles which have been forced in by the grafting-needle. A further source of error is a slightly lateral inoculation. Unless the skin and the abdominal wall are pierced exactly in the midline there is always the risk of a mammary tubule being involved in the puncture.

". . . In any case a failure to obtain growths in 90 per cent of the intraperitoneal experiments as against a success in 75 per cent of subcutaneous inoculations (where the chances of the virus encountering a mammary tubule are high but not even there 100 per cent) is strongly in favour of selective infectivity of the virus as against cell survival."

Thus, while "the technique of intraperitoneal inoculation is difficult, . . . the chances of the virus encountering a mammary tubule are high but not even 100 per cent" following subcutaneous inoculation.

Regarding the distribution of the mammary glands and the development of mammary cancer in males, Mann (33) stated: "In the male mouse, mammary tubules, imperfectly developed and without secreting acini, are present over large areas of the subcutaneous tissue, but even in high cancer strains male mice do not develop mammary cancer unless they are subjected to prolonged treatment with oestrin, when tumours occur as in the females—i.e., the virus changes from the latent to the active form."

These observations on the relative size of the mammary glands in male mice are not supported by details from other published reports. Gardner (24), in 1935, found that "The mammary glands of the normal male mouse persist as rudimentary ducts in the subcutaneous tissue throughout life. They undergo little, if any, growth after the weaning age." When male mice of various strains were from 6 to 9 weeks of age, Richardson and Cloudman (38) made whole mounts of their third glands. In males of the strains which showed the most extensive development, the mammary gland did not involve an area much greater than 1×1 cm. in area. Also, a gross dissection of a lactating female showed that intraperitoneal injection could be made without coming in contact with the mammary glands (11).

Twenty-one animals, eighteen males and three females, were tested by subcutaneous and intraperitoneal injections of a frozen suspension of the tumor-mince. Fourteen tumors were found in the subcutaneous region as against thirteen internal tumors. Although only a limited number of mice was tested, no difference was noted based upon the route of administration of the frozen tissue. In most experiments, Mann used only six animals, and her figures represent many series with several different tumors.

In 1949, Mann (33) tested the transplantable carcinoma "63" and determined that the inoculation of a frozen suspension was active in 19 (44 per cent of the mice developed tumors) of 22 experiments, while negative results were obtained in 3 experiments because of technical errors. The year previously, Dmochowski (22) assayed the carcinoma "63" for the mammary tumor milk agent and was unable to demonstrate the presence of the agent in desiccated tissue of tumors from the same laboratory. The tumors were from the 428th passage. No mention was made by Mann of these negative results by an accepted method of testing for the agent.

The usual method of reporting scientific data is to refer in the manuscript to any previous results other investigators have observed in that particular field. As this was not followed by either Gye (28) or Mann (33–35), it seems probable that they proposed that the reader should accept their theories as being original with them.

The interpretation of the experimental results following the use of either frozen and/or dried tumor tissue is dependent upon whether or not some cells may survive. In reporting their preliminary data and advancing the viral theory, Gye (28) made no direct reference to the previous studies of others, but, however, concluded that "We think we are justified in making the contention that the negative results of the past are merely negative and have the value of negative experiment which is the result of imperfect technique."

In a recent publication, Blumenthal and Walsh (12) referred to fifteen manuscripts, published previously to 1949, on the successful transfer of tumor and normal tissues following storage at low temperatures for as long as 2 years. The subject has also been reviewed by Hirschberg and Rusch (30). Only a few of these experiments, selected as pertinent to the discussion, will be considered here.

In 1939, Mider and Morton (36) froze mouse sarcoma and rat carcinoma to a minimum temperature of $-74°$ C. by using approximately the same technic as the British workers. When the tissue was frozen en masse, tumors developed following the subcutaneous inoculation of the thawed tissue. Repeated freezing and thawing reduced the number of tumors induced by the material. Among the three tumors they tested, sarcoma was more resistent to cold when frozen en masse than carcinoma, whereas, when frozen in a saline suspension, the opposite was found. If rat skin was maintained for 24 hours at a temperature of $-74°$ C. and transplanted subcutaneously, Mider and Morton

(36) found that when it was removed 10 days later there was histologic evidence of growth, as demonstrated by mitotic figures, characteristic staining of the connective tissue, etc.

Blumenthal and his associates (12, 13) concluded that normal tissue (thyroid and parathyroid of the guinea pig) and mouse Sarcoma 37 would survive freezing to either −70° C. or −190° C. and could later be successfully transplanted. The most accurate indicator of viability, mitotic activity, was noted in the transplants. In referring to the conclusions of Gye *et al.*, they stated that their "findings do not disprove the possibility of a viral transmission of neoplastic disease but rather prove that the criteria used for determining whether or not this mode of transmission obtains must be more strictly defined."

Craigie (16) reviewed the literature in a later report, the first paper in this series to do so, and stated: "Much of the experimental work reported on the production of tumours with frozen and thawed material is concerned with the interpretation of the survival of activity, and it is clear that under certain conditions malignant cells and normal skin . . . will survive freezing temperatures for some time." He tested various solutions to determine the ability of the cells to survive and concluded: "The degree of survival of activity in dextrose preparations at a low temperature is adequate for experimental work requiring the use of transplantable tumours and for some purposes may offer a more convenient source of material than fresh tumour tissue." Elsewhere, Craigie (17) wrote: "It is evident from the results of this experiment that the degree of survival of the different tumours varied considerably. Some lots produced tumours at all sites injected, others only some. . . . Complete failure to survive freezing has been observed so far only with two benzpyrene tumours mentioned and an undifferentiated tumour . . . arising near the point of needle entry of intraperitoneal injection of methylcholanthrene."

Mider and Morton (36) dried at least one tumor, while it was in the frozen state, in a lyophile apparatus and determined that the subcutaneous injection of the dried material did not produce tumors at the site of inoculation. Although the mammary tumor milk agent will remain active following desiccation (8, 19–23), the subcutaneous injection of fresh, dried tumor tissue has not "induced" mammary cancer at the site of injection within a matter of days or weeks; but after several months the typical spontaneous mammary tumors develop. These tumors appear with the usual random distribution seen for this type of cancer in mice. Similar results have been obtained following oral administration of an extract of lyophilized mammary cancer (frozen to −72° C. and dried *in vacuo*) (6).

Gye (28) reported that when fresh sporadic tumors from animals of the R3 and C3H stocks were dried, with the Craigie desiccator (15), active dry tissue was obtained. The type of tumor which they used was not specified, but these strains have a high incidence of spontaneous mammary cancer. However, until this new apparatus was employed, Gye and his associates (29) had been unsuccessful, in many experiments, in the propagation of dried tumor tissue, or "A long experience of negative results with a different and simpler drying method" (18).

Craigie (17) discussed the survival of cancerous cells following drying in their new apparatus as follows: "Tests of dried material obtained at various times during the development of the drying equipment (Craigie [15[1]]) were negative, although one positive result was obtained with C3H sarcoma and others were obtained by Gye, Begg, Mann, and Craigie (29[1])." In a later report (18) he concluded: "It would therefore seem to be reasonable to accept the possibility that a few cells (about 1 in 1,000,000) survived the drying process." Presumably, the number of cells to survive drying with their new equipment was sufficient to produce transplantable tumors at the site of inoculation. If this new desiccator did not completely destroy all the tumor cells, the tumors which arose at the site of inoculation of the partially dried material would be due to the propagation of these viable cells and not due to the action of any virus.

In view of the many experiments of others showing that tissues would survive freezing, it is interesting to cite from the 1947–48 report of Gye (27) as Director of the Imperial Cancer Research Fund: "Forty years ago Salvin-Moore successfully transplanted mouse carcinomata and sarcomata after they had been exposed to the temperature of liquid air. Cramer grew sarcomata after freezing them with CO_2 snow, but felt that his results were attributable to the survival of a few cells. Gye and Purdy (1931) studied the matter further and concluded that a few cells were able to remain viable though exposed to freezing temperatures. The subsequent work of Breedis and Furth (1938) and others has produced additional evidence that such survival may occur. Breedis and Furth drew attention to the value of freezing and storing on CO_2 snow as a method of preserving tumours, but although this method has been used by some it has not been generally adopted. Dr. Craigie is studying certain aspects of the problem

[1] References numbered as in this paper.

that do not seem to have been explored, with the immediate objective of further improving this potentially valuable method of preserving certain transplantable tumours."

Let us now refer to the papers published by Salvin-Moore and his associates (39–40) in *1908* which were mentioned by Gye (27) in 1947–48. Salvin-Moore exposed mouse tumors to the action of liquid air for periods of from 20 minutes to ½ hour. "They were then at once introduced into healthy mice beneath the skin, the presumption being that in these circumstances the tumour cells would be destroyed by the action of the liquid air and consequently that they would multiply no further." However, in some cases new tumors arose at the site of inoculation of the frozen tissue.

Salvin-Moore and Walker (40) discussed their results as follows: "From these observations it is rendered clear that exposure to liquid air at a temperature of about −195° does not necessarily destroy the potentiality of the substance of a mouse tumour to produce fresh tumours of the same kind in mice into which such frozen tumour substance has been grafted.

"These facts in themselves are somewhat surprising, and they immediately raise a number of questions which it will be desirable to have elucidated in the interests of research concerning the nature of cancer. In the first place, it is rendered clear that exposure to liquid air for a certain period of time does not destroy the principle upon which the vitality of mouse cancer depends. If, as may be the case, the cells composing the mass of the tumour, and constituting the grafts, are killed by exposure to liquid air, then the development of mouse cancer after such exposure indicates not merely that the growth of similar tumours is dependent on the integrity of the 'cancer cells,' but also that the new tissues are not necessarily formed from the implanted cells at all, and may arise from the cells of the new host in response to some stimulus introduced along with the frozen material, and quite independent of the integrity of the so-called 'cancer cells.' "

The authors (40) furthermore stated that their observations suggested "that the production of new tumours in the hosts into which the frozen cancer tissue has been introduced may possibly not be dependent upon the introduction of the 'cancer cells' at all, but upon the action of a virus which is independent of these cells, and retains its activity after being subjected to the temperature of liquid air." Salvin-Moore and Walker recognized the possibility that "it is not certain that the cells from the tumour introduced into a new individual are killed by half an hour's exposure to the temperature of liquid air, particularly as the seeds of some plants and trypanosomes are said to survive this temperature."

Earlier in this discussion we quoted a statement by Gye (28) "that the negative results of the past are merely negative and have the value of negative experiment which is the result of imperfect technique." These positive results were probably obtained, as demonstrated by Craigie (18), because of imperfect technic for the dehydration of cancerous tissue, either fresh or frozen. The tumors produced following the inoculation of suspensions of frozen tumors were likewise the result of the propagation of viable tumor cells. These data were incorrectly interpreted by the authors regardless of the published reports of many workers to the contrary.

Mider and Morton (36) commented upon the virus hypothesis of Salvin-Moore as follows: "This, however, has no bearing on the moot point concerning the possible relationship to transplantable tumors." There is little doubt but what this remark is as valid today for the inoculation of frozen tumor tissue as when it was made in 1939, and it applies equally well to the 1949 theory of Gye and Mann, since their explanation is practically identical with the one advanced by Salvin-Moore in 1908.

It has often been said that many theories are advanced only for the stimulus they may give to others for further research. Perhaps in some cases this may result in an increase in our knowledge of the problem being investigated, while in other instances it may actually retard progress until the hypothesis has either been refuted or confirmed. This may be necessary, especially when the experiments are completed without adequate controls.

SUMMARY

Salvin-Moore, in 1908, determined that tumors would develop in mice following the subcutaneous implantation of neoplasms which had been exposed to the temperature of liquid air (−195° C.). He proposed a virus theory to explain the results, although he considered the possibility that some cells had remained viable.

In 1949, Gye and his associates submitted a similar theory based upon observations obtained following the freezing and drying of mouse cancers. Furthermore, Mann stated that when mammary cancers (both transplanted and spontaneous) were frozen, the milk agent virus was liberated in its "active" form and when injected subcutaneously, mammary tumors would be produced at the site of injection within a short interval of days or weeks. Few mammary tumors arose at

other sites of inoculation because of the "selective infectivity" of the virus for mammary tissue.

To test for the active mammary tumor milk agent virus, transplanted mammary tumors were minced, suspended in dextrose, and frozen to −79° C. The thawed tumor-mince produced mammary tumors at the site of injection only in mice of stocks which would be susceptible to fresh tissue grafts of the tumor being tested. Negative results were obtained with mice of other strains, although they were susceptible to the development of spontaneous mammary cancer. Intraperitoneal inoculation of the frozen material was as effective as subcutaneous injection in the production of mammary tumors.

Whereas cell-free centrifugates have been found to contain the mammary tumor milk agent, cell-free extracts of the frozen mammary tumor-mince failed to induce tumors, within the specified interval, in any animal regardless of its genetic constitution.

Preliminary data indicate that the milk agent was no more active in cell-free extracts of the frozen than the fresh tissue of the same tumor, regardless of the route of administration. The final results of these tests will not be apparent for months, but the earliest tumors to be observed developed in mice which had received intraperitoneal injections.

The revival of this viral theory for mouse cancer by Gye *et al.* and Mann disregarded the previous finding of many investigators that mouse tissues (normal and cancerous) survived freezing to low temperatures. Evidence was also cited that the newly designed desiccator, used for their studies, did not destroy all cells. The fact that other methods of dehydration, including their own studies, had given negative results which would refute the virus hypothesis was disregarded as due to imperfect technic.

The experimental data submitted by Gye *et al.* and Mann were, without a doubt, based upon observations which they either incorrectly interpreted, as in the case of the studies with frozen tissues, or secured by using new equipment before it had been adequately tested and which resulted in imperfect technic.

REFERENCES

1. ANDERVONT, H. H., and BRYAN, W. R. Properties of the Mouse Mammary Tumor Milk Agent. J. Nat. Cancer Inst., **5**:143–49, 1944.
2. BITTNER, J. J. A Genetic Study of the Transplantation of Tumors Arising in Hybrid Mice. Am. J. Cancer, **15**: 2202–47, 1931.
3. ———. Genetic Studies on the Transplantation of Tumors. VII. Comparative Study of Tumors 19308 A, B, and C. *Ibid.*, **17**:724–34, 1933.
4. ———. Some Possible Effects of Nursing on the Mammary Gland Tumor Incidence in Mice. Science, **84**:162, 1936.
5. ———. "Influences" of Breast-Cancer Development in Mice. Pub. Health Rep., **54**:1590–97, 1939.
6. ———. The Preservation by Freezing and Drying in Vacuo of the Milk-Influence for the Development of Breast Cancer in Mice. Science, **93**:527–28, 1941.
7. ———. The Milk Influence of Breast Tumors in Mice. *Ibid.*, **95**:462–63, 1942.
8. ———. Inciting Influences in the Etiology of Mammary Cancer in Mice. A.A.A.S. Research Conference on Cancer, pp. 63–96. 1945.
9. ———. The Causes and Control of Mammary Cancer in Mice. Harvey Lectures, **42**:221–46, 1946–47.
10. ———. The Transplantability of Mammary Cancer in Mice Associated with the Source of the Mammary Tumor Milk Agent. Cancer Research, **7**:741–45, 1947.
11. ———. Some Enigmas Associated with the Genesis of Mammary Cancer in Mice. *Ibid.*, **8**:625–39, 1948.
12. BLUMENTHAL, H. T., and WALSH, L. B. Survival of Guinea Pig Thyroid and Parathyroid Autotransplants Previously Subjected to Extremely Low Temperatures. Proc. Soc. Exper. Biol. & Med., **73**:62–67, 19 0.
13. BLUMENTHAL, H. T.; WALSH, L. B.; and GREIFF, D. Studies on the Effect of Low Temperatures on the Transplantability of Normal and Neoplastic Tissue. Cancer Research, **10**:205, 1950.
14. CRAIGIE, J. A Pressure Mincer for the Preparation of Tumour Suspensions. Brit. J. Cancer, **3**:249–50, 1949.
15. ———. A Drying Apparatus for the Study of Tumour Transmission. *Ibid.*, pp. 250–55, 1949.
16. ———. The Preservation of Suspensions of Tumour Cells in Dextrose at Low Temperatures. *Ibid.*, pp. 268–74, 1949.
17. ———. A Quantitative Approach to the Study of Transplantable Tumours. Brit. M. J., **2**:1485–91, 1949.
18. ———. Director's report. 47th Annual Report, Imperial Cancer Research Fund, pp. 5–18. 1949–50.
19. DMOCHOWSKI, L. Mammary Tumour-inducing Factor and Genetic Constitution. Brit. J. Exper. Path., **25**:138–40, 1944.
20. ———. Comparative Potency of the Mammary Tumour Agent of Mice of Different Genetic Constitutions. *Ibid.*, **26**:267–69, 1945.
21. ———. Age and Dosage in the Induction of Breast Cancer in Mice by the Mouse Mammary Tumour Agent. *Ibid.*, pp. 192–97, 1945.
22. ———. Mammary Tumour-inducing Factor and Genetic Constitution. Brit. J. Cancer, **2**:94–102, 1948.
23. ———. Survival of the Milk Factor in a Transplantable Breast Tumour in Mice. *Ibid.*, **3**:246–48, 1949.
24. GARDNER, W. U. The Effect of Ovarian Hormones and Ovarian Grafts upon the Mammary Glands of Male Mice. Endocrinology, **19**:656–67, 1947.
25. GYE, W. E. Director's report. 42d Annual Report, Imperial Cancer Research Fund, pp. 5–10. 1944–45.
26. ———. Director's report. 43d Annual Report, Imperial Cancer Research Fund, pp. 7–13. 1945–46.
27. ———. Director's report. 45th Annual Report, Imperial Cancer Research Fund, pp. 5–13. 1947–48.
28. ———. The Propagation of Mouse Tumours by Means of Dried Tissue. Brit. M. J., **1**:511–15, 1949.
29. GYE, W. E.; BEGG, A. M.; MANN, I.; and CRAIGIE, J. The Survival of Activity of Mouse Sarcoma Tissue after Freezing and Drying. Brit. J. Cancer, **3**:259–67, 1949.
30. HIRSCHBERG, E., and RUSCH, H. P. Comments on Recent Experiments with Frozen and Dried Tissue as Evidence for the Virus Etiology of Tumors. Cancer Research, **10**: 335–38, 1950.

31. Little, C. C., and Strong, L. C. Genetic Studies on the Transplantation of Two Adenocarcinomata. J. Exper. Zoöl., **41**:93–114, 1924.
32. Mann, I. A Study of Cell Survival in Embryonic Tissue Grafts in Inbred Strains under Various Conditions. Brit. J. Cancer, **3**:255–59, 1949.
33. ———. Effect of Low Temperatures on the Bittner Virus of Mouse Carcinoma. Brit. M. J., **2**:251–53, 1949.
34. ———. Effect of Repeated Freezing and Thawing on Mouse Carcinoma Tissue. *Ibid.*, pp. 253–55, 1949.
35. Mann, I., and Dunn, W. J. Propagation of Mouse Carcinoma by Dried Tumour Tissue. Brit. M. J., **2**:255–57, 1949.
36. Mider, G. B., and Morton, J. J. The Effect of Freezing *in Vitro* on Some Transplantable Mammalian Tumors and on Normal Rat Skin. Am. J. Cancer, **35**:502–9, 1939.
37. National Cancer Institute, Staff. A Symposium on Mammary Tumors in Mice. A. A. A. S. 1945.
38. Richardson, F. L., and Cloudman, A. M. The Mammary Gland Development in Male Mice at Nine Weeks of Age. Anat. Rec., **97**:223–37, 1947.
39. Salvin-Moore, J. E., and Barratt, J. O. W. Note upon the Effect of Liquid Air upon the Graftable Cancer of Mice. Lancet, **1**:227, 1908.
40. Salvin-Moore, J. E., and Walker, C. E. On the Relationship of Cancer Cells to the Development of Cancer. Lancet, **1**:226–27, 1908.

Nuclei from Normal and Leukemic Mouse Spleen

I. The Isolation of Nuclei in Neutral Medium*

ROSE M. SCHNEIDER, M.S., AND MARY L. PETERMANN, PH.D.

(From the Sloan-Kettering Institute for Cancer Research, New York, N.Y.)

In the course of a study of the pentosenucleic acid (PNA) distribution in normal and leukemic mouse spleen, it was found desirable to determine the nucleic acid content of isolated nuclei. The procedures commonly employed for the isolation of nuclei (1, 4, 8, 13) involve the use of citric acid. This was felt to be unsuitable for the isolation of nuclei free of cytoplasmic PNA, since it has been shown (2) that the cytoplasmic nucleoproteins flocculate below pH 6.0 and precipitate at pH 4.8 or lower. The danger of contamination is particularly great in leukemic spleen, where the cytoplasmic PNA concentration is extremely high (9). Although the preparation of nuclei at pH 6.0 (4) might have been feasible, a procedure which avoided the use of acid entirely seemed more desirable.

When attempts were made to isolate nuclei in the 0.88 M sucrose medium introduced by Hogeboom, Schneider, and Pallade (6) for the isolation of mitochondria, the nuclei were clumped, distorted, and badly contaminated with debris. A search was therefore made for some ion which would "harden" the nuclei without causing agglutination of cytoplasm. Of the various salts tried, those containing divalent cations appeared most satisfactory; equally good results were obtained with calcium chloride and manganese sulfate. In the procedure described below, 0.0018 M calcium chloride in 0.88 M sucrose was used. The method is simple, convenient and rapid, being complete within 1 hour of the death of the animals.

MATERIALS AND METHODS

The isolation procedure outlined below was used for normal spleen only. Leukemic spleen will be discussed in the second paper of this series.[1] The mice, of the Akm strain, were sacrificed by spinal fracture. Their spleens were excised rapidly, chilled on ice, and cleaned free of visible fat. They were then weighed in 1-gm. batches (eight to twelve spleens per batch).

The entire procedure was carried out at about 4° C. Each gram of tissue was suspended in 15 cc. of 0.88 M sucrose containing 0.0018 M calcium chloride. The tissue was crushed in a glass homogenizer (inside diameter, 1 inch) by means of five strokes of the tube, with slight rotation.

The connective tissue was removed by straining, first through a double layer of surgical gauze, then through one layer of single-napped cotton flannelette, and finally through one layer of double-napped cotton flannelette into a 50-cc. centrifuge tube with short conical bottom, containing 0.05 cc. of isocaprylic alcohol.

The centrifuge tube was placed in a beaker of crushed ice and water and its contents homogenized for 5 minutes. The homogenizer consisted of a notched stainless steel blade, 1.4 cm. in diameter, attached to a shaft which was rotated by a Dumore motor rated at 16,000 r.p.m.

The homogenate, which was at pH 7.3, was transferred by capillary pipette to a 15-cc. graduated centrifuge tube and centrifuged for 10 minutes at 600 *g* in a horizontal head. The supernatant fluid was carefully decanted and the sediment mixed by capillary pipette with 2 cc. of the medium, until the suspension was visibly uniform. The suspension was then diluted to 10 cc. and centrifuged again at 600 *g* for 10 minutes. The washing was repeated twice in this manner. The last sediment was suspended in 2 cc. for microscopic examination, then diluted to 5 cc. for subsequent counting.

Wet smears stained routinely with methyl green–pyronin were examined microscopically under the oil immersion objective, at a total magnification of 1,225. Nuclei could be clearly differ-

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entiated by their blue color, which contrasted sharply with the pink of cytoplasm and connective tissue. Red blood cells remained unstained. The orcein-fast green stain of Kurnick and Ris (7) served as a confirmatory stain.

A sample of the 5-cc. suspension was taken up in a red blood cell pipette, diluted 1:100 with the medium, and counted in a haemocytometer. The contents of the entire square millimeter area commonly allotted to the red cell count were totaled, and the result multiplied by 10^6 to give the number of nuclei per cubic centimeter of suspension. The count per cubic centimeter ranged from 50 to 100 million nuclei.

One-cc. samples of the final suspension were analyzed for DNA and PNA by the modified Schneider (11, 12) procedure previously described (9). Total nitrogen was determined by semi-micro Kjeldahl analysis. From these values the amount per nucleus was calculated.

RESULTS

Microscopic examination of the final sediment revealed isolated nuclei, free of cytoplasmic tags or connective tissue. They appeared round, unclumped, and undistorted, and resembled morphologically those seen in fixed sections of mouse spleen. Polymorphonuclear nuclei, with their characteristic segmentations, appeared equally free of cytoplasm. Infrequent whole cells were readily identified under immersion oil by the presence of a thin rim of pyronin-stained cytoplasm. Differential counts showed whole cells in a frequency of 2 per cent or less. Phase-contrast photomicrographs of unstained nuclei suspended in the sucrose-calcium chloride medium are shown in Figure 1.

The results of the chemical analyses are shown in Table 1. The PNA content per nucleus, 0.29 $\times 10^{-9}$ mg., is less than 5 per cent of the DNA content. The determination of such a small amount of PNA by the orcinol reaction, in the presence of a great excess of DNA, is very difficult, and extreme care must be taken to get reproducible results. The greater variability of the PNA values, as compared to the DNA and nitrogen values, probably reflects this technical difficulty.

TABLE 1

THE NITROGEN AND NUCLEIC ACID CONTENT OF MOUSE SPLEEN NUCLEI

	Nitrogen*	PNA*	DNA*
Av.†	3.6	0.29	6.5
Range	3.3–4.0	0.22–0.37	5.9–7.1

* Mg. per nucleus $\times 10^9$.
† Seven experiments.

DISCUSSION

No attempt was made to investigate the mechanism by which the morphological integrity of the nuclei was maintained in the calcium-sucrose medium. The concentration of calcium chloride chosen was just enough to prevent stickiness and yet yield the greatest number of isolated nuclei. The optimal concentration appeared to be quite critical, since greater amounts of salt rendered the whole cells resistant to fragmentation. The same medium was also found to be the best diluent for the counting procedure.

Homogenization in the absence of an antifoaming agent resulted in microscopically transparent pouchlike structures, presumably air bubbles, whose surface was coated with cytoplasm. The elimination of foaming prevented contamination of the nuclei with these structures and reduced the time required for homogenization. Isocaprylic alcohol was the most satisfactory of the agents

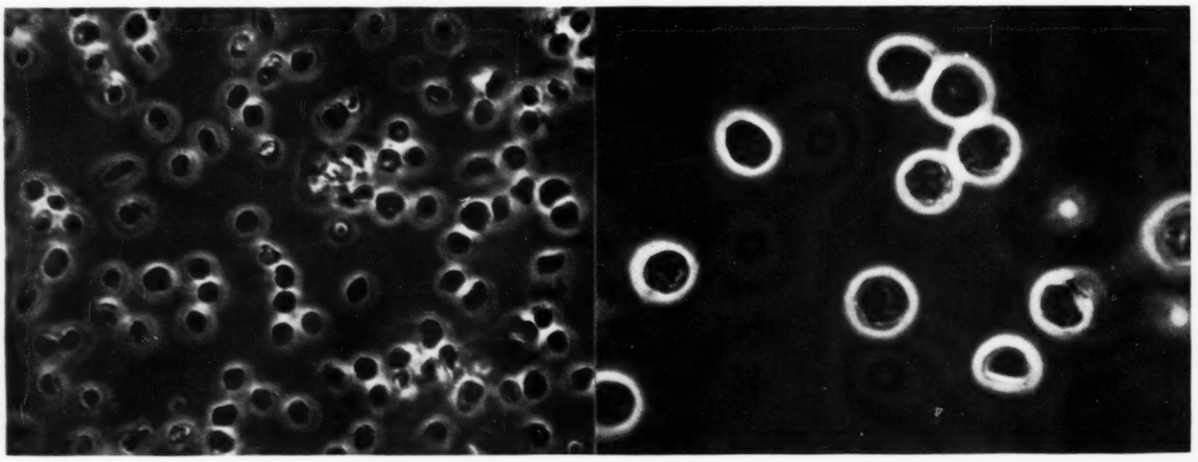

FIG. 1.—Isolated nuclei from mouse spleen photographed by phase-contrast microscopy. *Left*, high dry, mag. × 540; *right*, oil immersion, mag. × 1,210.

tried. The amount of alcohol, however, should be minimal; excessive amounts result in accumulation of the nuclei, together with cytoplasmic contaminants, in a surface pellet during centrifugation.

Although a less brisk homogenization might have been sufficient to disrupt cell membranes (6), the type of homogenization employed was selected because of its rapid fragmentation of the red blood cells. The final nuclear sediment was therefore white, apparently free of hemoglobin.

The isolation procedure was worked out under microscopic control. Smears made subsequent to crushing the whole spleens showed an abundance of whole lymphocytes, nuclei, red blood cells, non-nuclear debris, and fine fibers. During the filtrations the fibers were removed, held back on the nap of the flannelette. The homogenate consisted of nuclei and non-nuclear debris, with a conspicuous lack of whole cells and red blood cells. The final sediment was composed almost entirely of nuclei, as described above.

It is to be expected that the DNA content per nucleus would be independent of the method of isolation, while the PNA and nitrogen content per nucleus would vary with the completeness of removal of the cytoplasm. This is borne out by the analytical results. The DNA per nucleus, 6.5×10^{-9} mg., agrees with the values reported for mouse (1) and rat (3) spleen nuclei isolated in citric acid and is close to the values reported for mouse liver and kidney (14). Attempts to determine DNA per nucleus on total spleen homogenates have so far been unsuccessful. When spleen is homogenized before removal of the connective tissue, microscopic examination shows clusters of nuclei trapped in networks of fibers. Nuclear counts on this material are therefore not reliable.

In contrast to the agreement in DNA, the PNA and nitrogen per nucleus reported here are very much lower than the values obtained on mouse spleen nuclei isolated in sucrose and dilute citric acid (1). While these low values may be the result of an extraction of nuclear PNA by the sucrose–calcium chloride medium, this seems unlikely, since the preparation is completed within 1 hour.

It may be calculated from the total DNA per gram of mouse spleen, 17 mg. (9), that 1 gm. of spleen contains about 2.6×10^9 nuclei. The range of $2.5–5.0 \times 10^8$ nuclei isolated from 1 gm. of tissue therefore represents a yield of 10–20 per cent.

It has recently been suggested (5, 10) that when nuclei are isolated in aqueous media, a large amount of protein is extracted from them in the course of the isolation. The protein to DNA ratio of the nuclei described in this paper is about 3.5, close to the values quoted for nuclei isolated by other procedures (10), and far lower than the ratios of 8–10 found by cytochemical methods. It can be calculated, however, from the N/DNA ratio of these nuclei, 0.55, that recovery of the entire 17 mg. of DNA in 1 gm. of spleen would yield about 9.4 mg. of nitrogen. This is 30 per cent of the total nitrogen, a reasonable value for a tissue of such a large nuclear volume. While such a calculation does not rule out small losses of protein, it suggests that losses of the magnitude indicated by Pollister and Leuchtenberger (10) probably do not occur.

Preliminary experiments indicate that the procedure described above is also applicable to the isolation of nuclei from calf thymus, an organ which is also rich in lymphocytes. For the isolation of nuclei from nonlymphocytic organs, modifications in batch size, calcium concentration, or time of homogenization may be necessary.

SUMMARY

1. A procedure is reported for the isolation of mouse spleen nuclei in a neutral medium consisting of 0.88 M sucrose and 0.0018 M calcium chloride. The recovery of nuclei ranges from 10 to 20 per cent.

2. The isolated nuclei appear to be morphologically intact and free of cytoplasm and connective tissue.

3. These nuclei contain 6.5 mg. of DNA, 0.29 mg. of PNA, and 3.6 mg. of nitrogen per 10^9 nuclei.

REFERENCES

1. ARNESEN, K.; GOLDSMITH, Y.; and DULANEY, A. D. Antigenic Properties of Nuclei Segregated from Spleens of Normal and Leukemic Mice. Cancer Research, **9**:669–71, 1949.
2. CLAUDE, A. Fractionation of Mammalian Liver Cells by Differential Centrifugation. II. Experimental Procedures and Results. J. Exper. Med., **84**:61–89, 1946.
3. CUNNINGHAM, L.; GRIFFIN, A. C.; and LUCK, J. M. Polyploidy and Cancer. The Desoxypentosenucleic Acid Content of Nuclei of Normal, Precancerous, and Neoplastic Rat Tissues. J. Gen. Physiol., **34**:59–63, 1950.
4. DOUNCE, A. L., and BEYER, G. T. The Arginase Activity of Isolated Cell Nuclei. J. Biol. Chem., **174**:859–72, 1948.
5. DOUNCE, A. L.; TISHKOFF, G. H.; BARNETT, S. R.; and FREER, R. M., Free Amino Acids and Nucleic Acid Content of Cell Nuclei Isolated by a Modification of Behrens' Technique. J. Gen. Physiol., **33**:629–42, 1950.
6. HOGEBOOM, G. H.; SCHNEIDER, W. C.; and PALLADE, G. E. Cytochemical Studies of Mammalian Tissues. I. Isolation of Intact Mitochrondria from Rat Liver; Some Biochemical Properties of Mitochondria and Submicroscopic Particulate Material. J. Biol. Chem., **172**:619–35, 1948.
7. KURNICK, N. B., and RIS, H. A New Stain Mixture: Aceto-Orcein-Fast-Green. Stain Technol., **23**:17–18, 1948.
8. MARSHAK, A. P_{32} Uptake by Nuclei. J. Gen. Physiol., **25**: 275–91, 1941–42.

9. Petermann, M. L.; Alfin-Slater, R. B.; and Larack, A. M. The Nucleic Acid Distribution in Normal and Leukemic Mouse Spleen. Cancer, **2**:510–15, 1949.
10. Pollister, A. W., and Leuchtenberger, C. The Nucleoprotein Content of Whole Nuclei. Proc. Nat. Acad. Sc., **35**:66–71, 1949.
11. Schneider, W. C. Phosphorus Compounds in Animal Tissues. I. Extraction and Estimation of Desoxypentose Nucleic Acid and of Pentose Nucleic Acid. J. Biol. Chem., **161**:293–303, 1945.
12. ———. Phosphorus Compounds in Animal Tissues. III. A Comparison of Methods for the Estimation of Nucleic Acids. J. Biol. Chem., **164**:747–51, 1946.
13. Stoneburg, C. A. Lipids of the Cell Nuclei. J. Biol. Chem., **129**:189–96, 1939.
14. Vendrely, R., and Vendrely, C. La teneur du noyau cellulaire en acide désoxyribonucléique à travers les organes, les individus et les espèces animales. Experientia, **4**:1–10, 1948.

Induced Tumors of the Parotid Gland*

WILLIAM H. BAUER, M.D., AND JOHN J. BYRNE, D.D.S.

(From the Department of Pathology, School of Dentistry, St. Louis University, St. Louis, Mo.)

This experimental work was undertaken to study the effects of various carcinogens on salivary glands in the hope of producing primary epithelial neoplasms, and, among these, the so-called "mixed tumors" of the parotid and submaxillary glands. Indeed, this study has been stimulated by the meager results obtained in this field by a few authors and the uncertainty concerning the origin and pathogenesis of these epithelial tumors of varied patterns. Additional work will be described at a later date on the transplantability of the neoplasms obtained and the tumor development, under various conditions, which altered the physiologic activity of the glands prior to and following the introduction of various carcinogens.

Löwenstein (8), in 1910, injected scarlet red oil into the parotid glands of rabbits and produced inflammatory changes which he considered responsible for atypical epithelial proliferation of the small ducts. He claimed that the changes resembled human cylindromas.

Macchiarulo and Büngeler (9), in 1929, obtained "squamous epithelial cysts" containing large masses of keratin as a result of repeated injections of tar into the parotid glands of 70 rats. The initial alterations involved the nucleus and cytoplasm of the epithelial cells.

Abb (1), in 1932, injected scarlet red with and without Peru balsam, tar, or kieselguhr into nine rats and five rabbits. These treatments brought about the formation of cysts surrounded by squamous epithelium with keratinization and metaplastic changes.

Benecke and Schröder (2) injected benzpyrene in olive oil into the parotid glands of 53 rats and 55 mice but obtained positive results in only two rats. These were sarcomas. The oil cysts which developed were lined by papillary epithelial proliferations with massive keratin.

In their experiments Franseen, Aub, and Simpson (4) introduced methylcholanthrene and 1,2,5,-6-dibenzanthracene into the salivary glands of 30 mice, producing 17 tumors, 12 of which were epidermoid and 1 a fibrosarcoma. These neoplasms, which developed in about 165 days, displayed cyst formation. No mixed tumors were obtained.

In 1940 Rusch, Baumann, and Maison (13) surgically exposed the submaxillary gland of mice and introduced 1,2,5,6-dibenzanthracene and 3,4-benzpyrene. In 2–3 months the resulting tumors measured about 2.5 cm. These authors described a metaplasia of the glandular epithelium to stratified squamous epithelium with occasional malignancy.

An investigation of the histogenesis of tumors of the salivary glands by Steiner (16) produced a comprehensive work on this subject. Into mice he inserted 1,2,5,6-dibenzanthracene and also cholesterol. Rats were treated with benzpyrene and methylcholanthrene and guinea pigs with methylcholanthrene and cholesterol. The site of deposition of the chemical was the submaxillary gland. Methylcholanthrene was used in a similar manner in rabbits. All animals except the rabbits responded with tumor production—usually squamous-cell carcinoma. No adenomas were produced. The rabbits developed an early epithelial metaplasia which failed to become malignant.

EXPERIMENTAL

Studies with Rabbits

Because of the specificity of species response, the type of animal, as well as the carcinogen, was altered several times. Although little work had been done on rabbits, the size and accessibility of the rabbit parotid gland prompted its investigation.

Rabbits weighing 4–5 pounds were selected for implantation with the following chemicals: methylcholanthrene, 9,10-dimethyl-1,2-benzanthracene, 1,2-benzanthracene, and 1,2,5,6-dibenzanthracene. The pellets for implantation were 5 mm. long, 19-gauge in cross section, and were composed of a 20 per cent solution of the carcinogen in beeswax. The pellets, usually 2 or 3, each weighing 1.2 mg., were introduced into the surgically exposed main duct and parotid gland proper from a small syringe. Twenty animals were treated with pellets in this way, but one death decreased the number

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to nineteen. In six animals, methylcholanthrene was the agent; in four, 9,10-dimethyl-1,2-benzanthracene, and in nine, 1,2-benzanthracene. The animals were killed at intervals of 3 months to 1½ years. The parotid glands, submaxillary glands, kidneys, liver, and spleen were removed for microscopic studies. Subserial sections were stained with hematoxylin and eosin.

Results.—No malignant tumor was observed in animals maintained for 1½ years. Two types of response were recognizable: one of inflammation, associated with extensive degenerative changes but without epithelial proliferation, the other of moderate inflammation with proliferation of ductal epithelium. Inflammatory edema destroyed the structural arrangement of the parotid gland and of the adjacent lymph nodes. Prominent findings were periductal round-cell infiltration and fatty and hyalin degeneration. The almost universal fibrosis in cases of long standing was grossly visible; it replaced portions of the gland and surrounding tissue and often showed areas of central liquefaction.

In other instances, the tissue in the vicinity of the pellets, which remained intact for many months, frequently revealed hyalinization. The hyalin appeared altered in its staining reaction by combination with the secretion of epithelial cells, the nuclei of which were scattered through this pink and bluish stained area. Eosinophiles and plasma cells were usually observed. There was proliferation of ductal epithelium together with the formation of a moderate number of new ducts which in some areas were expanded and lined by several layers of epithelial cells. This feature bears a definite resemblance with the pseudo-cartilage of the so-called "mixed tumors" of the salivary glands (Fig. 1). In a few sections, growth of adjacent lymphoid tissue into the glands was observed.

Studies with Mice

In view of the resistance of the rabbit to production of epithelial tumors of the salivary glands, further experiments were carried out with mice, the following strains being used: C57 black, C(b alb c), A, and AK. The carcinogens used were: 1,2,5,6-dibenzanthracene, methylcholanthrene, and 9,10-dimethyl-1,2-benzanthracene.

The experiment consisted of implanting 0.3 mg. of 20 per cent carcinogen in beeswax (four 20-gauge pellets 3 mm. long) into surgically exposed parotid and submaxillary glands.

Table 1 indicates the number of mice of the four strains treated with the various carcinogens.

Results.—Although the response to the carcinogens was characteristic to the strain, there was no relationship between the strain and the types of tumors produced by the chemical. Initial tumor production occurred simultaneously in C57, A, and C strains at about 14 weeks. However, in later attempts with about one-half the dose of carcinogen, the C type animals responded as early as 9 weeks.

The C57 black strain had a longer induction period, requiring 1–2 months longer for the relatively few tumors produced, and it was also considerably more sensitive to the toxic action of the agents.

The response of strain C was the most promising. The neoplasms presented an extraordinary variety of types of histologic patterns. Metastases to the lungs were observed in this group.

Although few strain A animals were used, they responded to all agents with early tumor production. Only one animal did not develop a malignant tumor by the third month.

TABLE 1

THE NUMBER AND STRAIN OF MICE TREATED WITH CARCINOGENS

Agent	C57	C	A	AK
1,2,5,6-Dibenzanthracene	8	5	2	
Methylcholanthrene	9	61	2	50
9,10-Dimethyl-1,2-benzanthracene	6	5	2	

Strain AK, still under observation, responded poorly even at 6 months, only three malignant neoplasms having developed in the 25 animals examined.

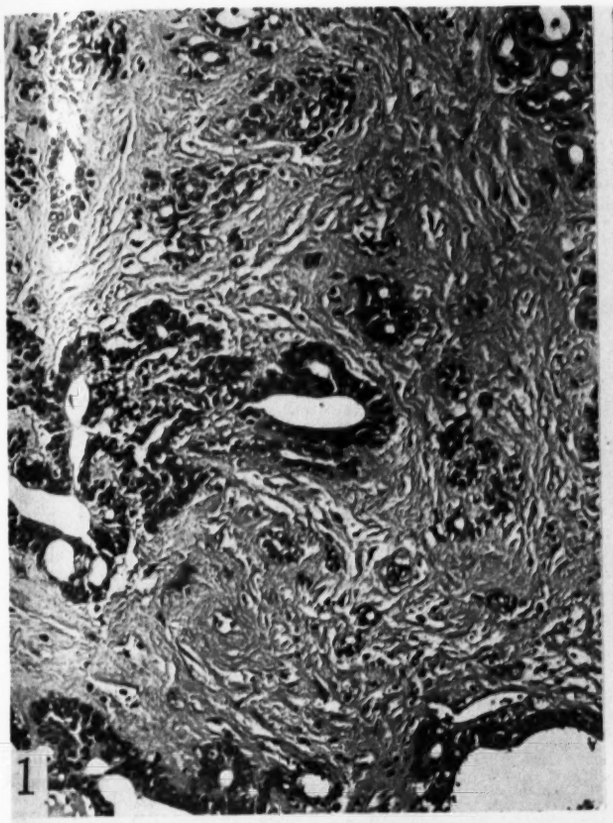
Macroscopically, all tumors resembled each other. The few tumors that appeared connected with or that had perforated the skin were discarded, to avoid the study of possible epidermal tumors. The tumors varied in size from 1 to 2 cm. or more in diameter. Some of them appeared encapsulated, others did not. The tumors were solid or cystic. The latter occasionally contained mucoid material.

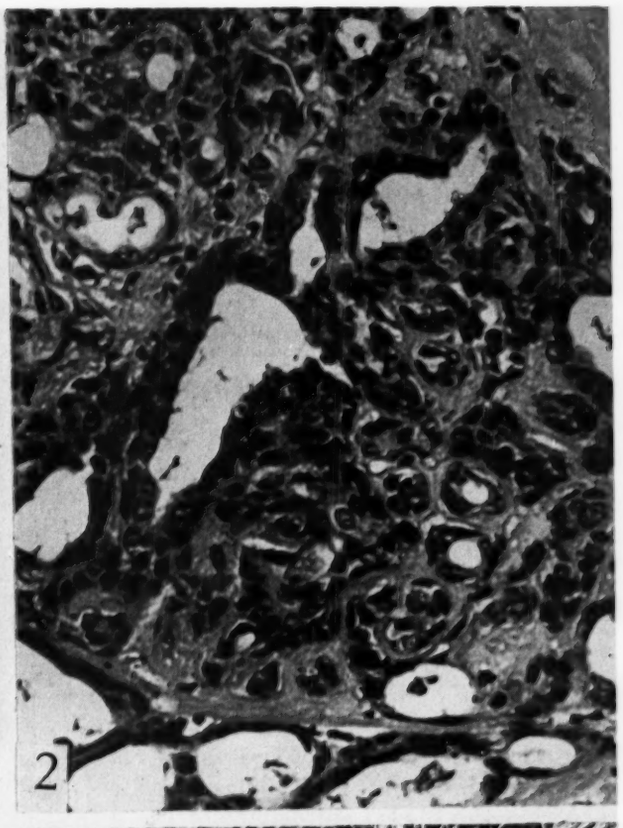
Microscopic Examination

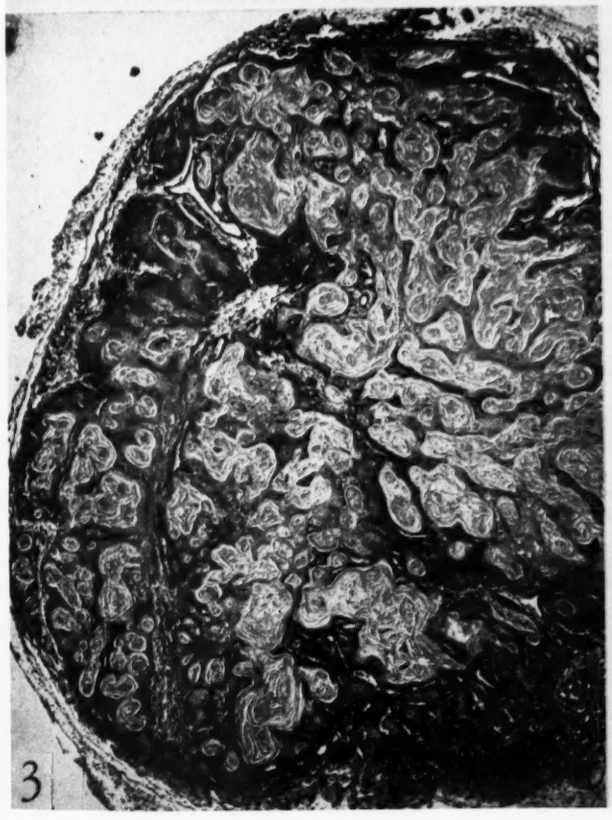
Since the produced neoplasms did not differ fundamentally in their histologic pattern, regardless of the strain or carcinogen which had been employed, an individual analysis of the microscopic details was deemed unnecessary. The following description is generally applicable to all our cases.

Although the reaction to the carcinogen pellets started around the implant, subserial sections evidenced a multicentric growth that followed the initial response.

The inflammatory alterations were usually moderate, and the hyalinization of the tissue adjacent to the pellet was not so pronounced as in some rabbits. However, even in the cases of small tumors, the tendencies toward proliferation of ductal







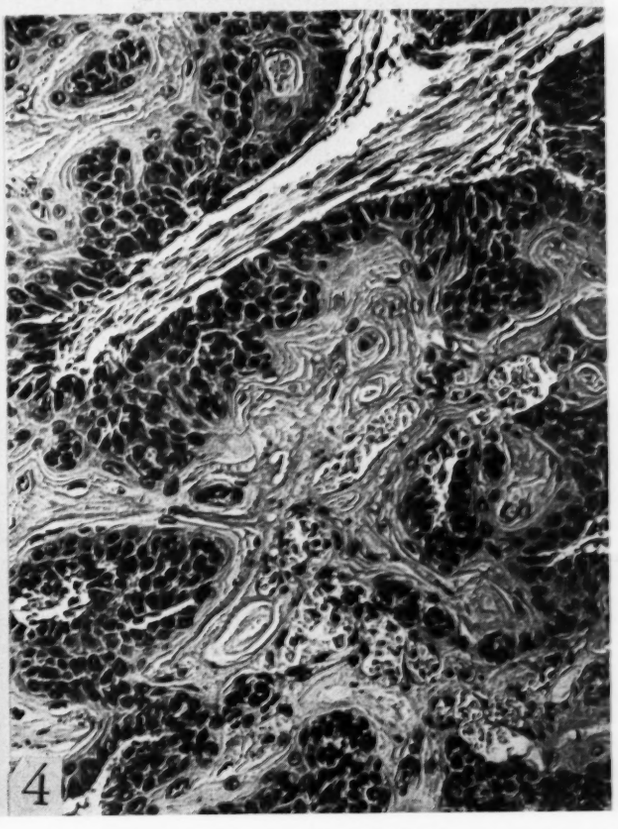


FIG. 1.—Rabbit parotid gland; 9,10-dimethyl-1,2-benzanthracene, 12-month duration. Proliferation of ductal epithelium and new duct formation in dense hyalinized cartilage-like tissue resembles so-called "mixed tumor" of salivary glands. × 120.

FIG. 2.—Mouse, strain C; 9,10-dimethyl-1,2-benzanthracene, 3-month duration; adenoma of parotid gland. × 200.

FIG. 3.—Mouse, strain C; methylcholanthrene, 9-week, duration; adenoacanthoma of parotid gland, 2 cm. in diameter. × 40.

FIG. 4.—Peripheral area of Figure 3. Large masses of whorled keratin bordered by cords of palisading polymorphous cells with hyperchromatic nuclei. × 250.

epithelium, transformation of its columnar cells into cuboidal or flattened epithelium, and formation of ductlike structures, lined by multi-layered epithelium, were noticeable. Participation of acinous cells in the response was uncommon.

This reaction, which was generally seen, was the initial stage of a great variety of morphologic features. In few cases (strain C with methylcholanthrene), we found adenomatous growths of low-grade malignancy (Fig. 2) composed of newly formed serous and few mucous acini with an admixture of tubules and solid aggregations of squamous epithelial cells. We traced the origin of these cells to the epithelium of the intercalated ducts which possesses a pluripotential plasticity. It has been established by Schaper and Cohen (14) and Skorpil (15), among others, that these columnar epithelial cells may differentiate into serous, mucous, and squamous epithelium. Our study of sections from 73 normal parotid glands, removed at random at the autopsy of adults, and from 134 primary epithelial parotid gland tumors corroborated not only this statement but also the findings of Hartz (6) and Meza-Chávez (12) that sebaceous glands may arise from the ductal epithelium of the salivary glands.

Most of the tumors in mice were adenoacanthomas which displayed papillary arranged epithelium with excessive keratinization and glandular structures. There was variation in the distribution of these features; in some instances, the pattern was that of a well differentiated acanthoma, while in others, however, less often, an adenoma prevailed. Some tumors of such a composition were frankly malignant, whereas others showed a low-grade malignancy, or none. A relatively small number of polymorphonuclear cells and lymphocytes was scattered throughout the epithelial and stromal tissue with some exudate in the lumina of the newly formed ductlike structures. Strands of proliferated cuboidal, columnar, and polyhedral epithelial cells of ductal origin with hyperchromatic nuclei encircled masses of whorled keratin into which cords of epithelium, with a dense fibrous-tissue center, extended. The keratin contained chromatin particles and pearl bodies (Figs. 3, 4). Ductlike structures surrounded by the epithelium described above were filled with sloughed cells and nuclear debris. Single cells also showed keratinization.

Since the transplantability of these tumors and the behavior of the transplants will be discussed in a separate study, it is sufficient to indicate that the adenoacanthoma took very well in the kidney and liver, maintaining its original structural features (Fig. 5).

There were also variants of this tumor which represented the features of a papillary cystadenoma and papillary cystadenocarcinoma. The papillae which contained either a very vascular loose or myxomatous connective tissue or hyalinized material were lined by single or multiple layers of cuboidal epithelium and projected into blood-filled cavities (Fig. 6). In many instances, the base of the papillary carcinomatous projections merged with collections of poorly differentiated, malignant epithelial cells.

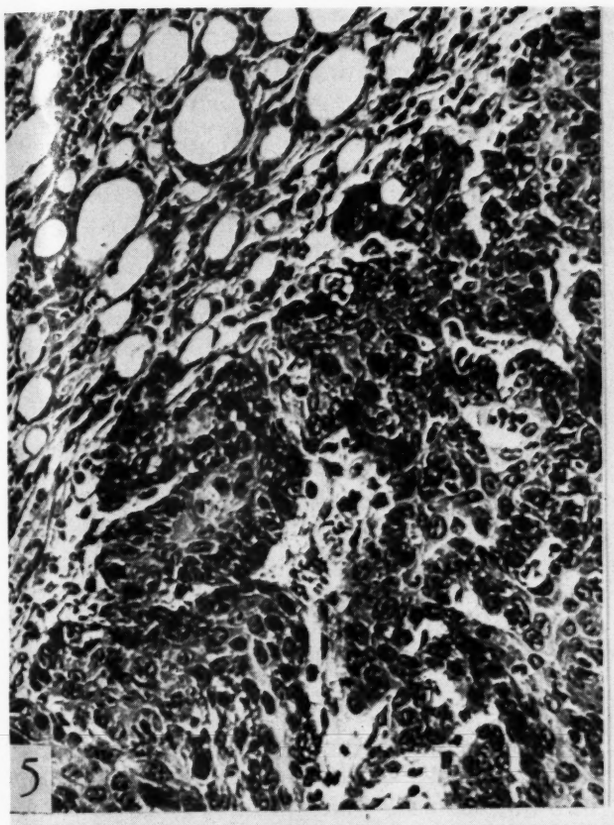
Another pattern of the tumor produced in a strain C mouse by 0.25 mg. methylcholanthrene in 4½ months displayed densely packed masses of vacuolated acinous cells with faint nuclei. Hyperchromatic nuclei, cells of fusiform, stellate, oval, and round shape were prominent between the vacuolated epithelial cells. We are inclined to regard them as myoepithelial cells within an adenocarcinoma with many mitotic figures (Fig. 7). It is of interest that there were metastases to the lungs.

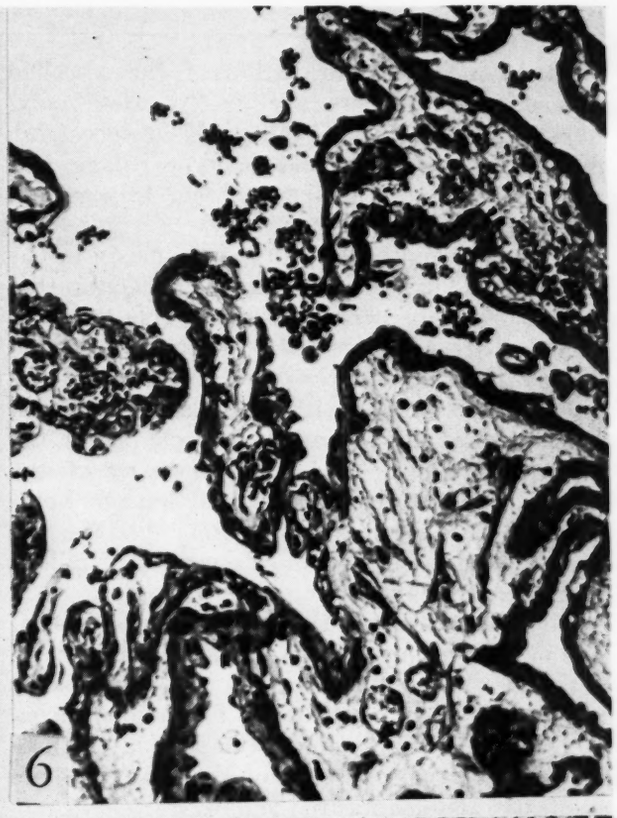
A small percentage of the neoplasms which were produced were diagnosed as rapidly growing, undifferentiated carcinomata with an occasional admixture of glandular formations. In some instances, these tumors were composed of cells with hyperchromatic nuclei and scant cytoplasm; in others, the cells appeared vacuolated. No squamous differentiation was detectable. Some of these undifferentiated carcinomata grew well when transplanted into the kidney and liver.

Quite a few rapidly growing, large neoplasms of the parotid glands of mice of the AK and C strains were composed of variably sized cells with round to oval hyperchromatic nuclei and many mitotic figures. Numerous, rather sharply bordered, multinucleated giant cells were scattered throughout the tissue. An occasional cell was large, elongated, and contained a very large hyperchromatic, irregular or lobulated nucleus. The parotid glands were completely replaced by this type of tumor which was partly hemorrhagic and necrotic. Many tumor cells had drawn out cytoplasmic processes, thereby resembling malignant fibroblasts. However, a thorough examination of subserial sections clearly demonstrated a gradual transition from epithelial cells to polyhedral and giant cells. This led the authors to interpret the tumor as a giant-cell carcinoma (Fig. 8). Microscopically, the thyroids were not involved.

DISCUSSION

While the introduction of various carcinogens into the parotid glands of rabbits did not produce true neoplasms, 58 tumors were obtained by the same methods in 150 mice of different strains. The





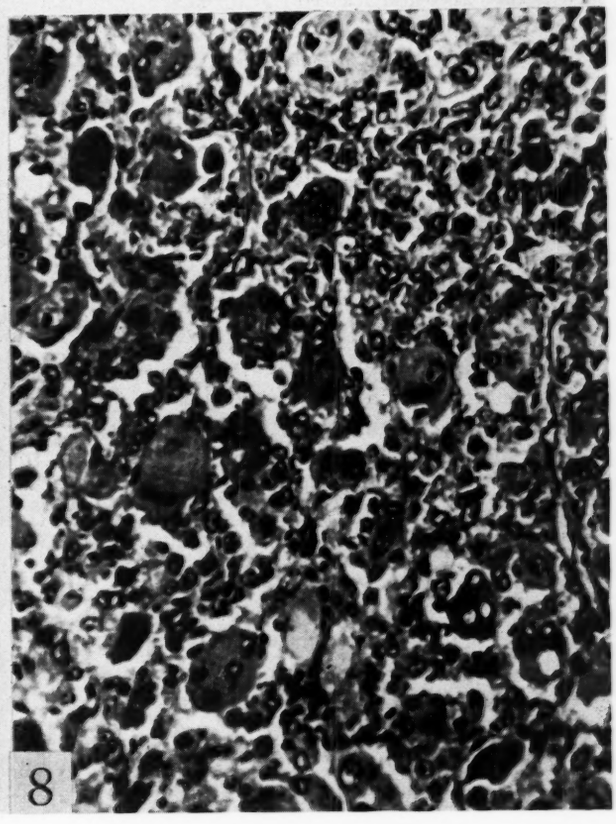


FIG. 5.—Transplant of adenoacanthoma (Fig. 4) growing in the kidney of a mouse, strain C. × 125.

FIG. 6.—Mouse, strain C; 9,10-dimethyl-1,2-benzanthracene, 11-week duration; papillary cystadenoma of parotid. × 125.

FIG. 7.—Mouse, strain C; methylcholanthrene, 4½-month duration; undifferentiated carcinoma of parotid gland with proliferated myoepithelial cells. × 256.

FIG. 8.—Mouse, strain C; methylcholanthrene, 20-week duration; parotid gland, giant-cell carcinoma. ×250.

effort to incite the formation of the so-called "mixed tumor"—more adequately called polymorphic adenoma (Willis [18])—was unsuccessful, although small areas of pseudo-cartilage and adenomatous structures resembling this growth were found in some of our tumors.

The most commonly induced neoplasm of the parotid gland in the mouse was the adenoacanthoma, occurring in varied patterns. This tumor is seldom observed in its classic form, that is, displaying the characteristics of keratinization of epithelial cells associated with glandular structures in the parotid glands of human subjects. While the amount of keratin was great in many of the growths, there were others with little or no keratin. In the latter instance, the tumors had the features of a capillary cystadenoma and capillary cystadenocarcinoma.

We observed the participation of myoepithelial cells in the formation of an adenocarcinoma with metastases in the lung showing the morphologic features of the primary tumor. The myoepithelial cells stained brilliant red with hämalaun-erythrosin-safranin (trichrome stain of Masson), while the connective tissue appeared yellow.

Adenoacanthomas of the salivary glands displaying many variations of structure recently received special attention in view of the work of McPeak and Arons (10) and McPeak and Warren (11) on adenoacanthomas in the esophagus and cardio-esophageal junction originating in the esophageal mucous glands, which are identical with the salivary glands.

The occurrence of rapidly growing parotid gland tumors in mice, showing an admixture of an adenoacanthoma with a sarcoma-like tissue containing numerous multinucleated giant cells, hemorrhages, and necrosis is of significance. The sarcoma-like tissue contained clearly bordered epithelial cells, occasional pearl bodies, and keratinized individual epithelial cells. Careful study of these tumors revealed a smooth transition of the cells of the solid acanthoma cords into elongated spindle-shaped cells and multinucleated giant cells. This finding induced us to conceive of these neoplasms as so-called giant cell carcinomata of the parotid glands. The thyroids were intact. The fact that these tumors have been produced by carcinogens does not diminish the importance of this finding. These growths were wholly of epithelial origin, even though collagenous fibers were demonstrable in the sarcoma-like part. Following the work of Foot and Day (3) the Tureen and Seelig (17), Hebbel (7) showed that the presence of collagenous fibers in the sarcomatous portions of an epithelial tumor of the thyroid does not interfere with the interpretation of this tumor as a giant-cell carcinoma. Hamperl (5) arrived at the same conclusion in his study of two "carcinosarcomas" of the breast but conceived of the spindle-shaped cells as myoepithelial cells, because their cytoplasm stained red with erythrosin. According to Willis (18), spindle cells and giant cells in a carcinoma result from the lack of differentiation. However, the morphologic structure of these giant-cell carcinomata differed considerably from the few poorly differentiated carcinomata which we produced. Spindle cells and giant cells were entirely absent from the latter.

We were not able to demonstrate that these induced tumors of the salivary glands of mice took origin from the acinous epithelium. However, we found evidence for the fact that they developed from the pluripotential epithelial cells of the intercalated ducts. This opinion agrees well with the so-called Heidenhain theory and with the study of Schaper and Cohen (14), who considered the cells of the intercalated ducts to be centers of proliferation. These cells may differentiate into squamous cells or, under neoplastic conditions, into mucous cells which are not found in normal parotid glands of adults.

SUMMARY

1. Rabbits seem to possess an unusual resistance to the production of induced salivary gland tumors.

2. Of the mice employed, strain C was the most responsive; strains A, C57 black, and AK, in that order, were less responsive.

3. Once the neoplasm had been induced, the strain of the mouse did not influence the type of tumor produced.

4. The adenoacanthoma with its many varied patterns was the most frequently produced tumor.

5. Related tumors were adenoma, papillary cyst adenoma, undifferentiated carcinoma, and giant-cell carcinoma. Metastases into the lungs occurred.

6. The site of origin of the initial metaplastic change and of the subsequent neoplasm was the pluripotential cuboidal epithelial cell of the intercalated ducts. These ducts may be regarded as proliferation centers.

REFERENCES

1. Abb, M. Zur experimentellen Erzeugung von Parotistumoren. Arch. f. Ohren-, Nasen- u. Kehlkopfh., **133**: 117–30, 1932.
2. Benecke, E., and Schröder, J. Über experimentelle Geschwulsterzeugung in Speicheldrüsen. Ztschr. f. Krebsforsch., **49**: 505–14, 1939.
3. Foot, N. C., and Day, H. A. The Occurrence of Reticulum in Tumors. Am. J. Path., **1**: 431–43, 1925.

4. FRANSEEN, C. C.; AUB, J. C.; and SIMPSON, C. L. Experimental Tumors in Lymphnodes and in Endocrine and Salivary Glands. Cancer Research, **1**:489–93, 1941.
5. HAMPERL, H. Über die Myothelien (myo-epithelialen Elemente) der Brustdrüse. Virchows Arch. f. path. Anat., **305**:171–215, 1939.
6. HARTZ, P. H. Development of Sebaceous Glands from Intralobular Ducts of the Parotid Gland. Arch. Path., **41**: 651–54, 1946.
7. HEBBEL, R. Giant Cell Carcinoma of the Thyroid. Arch. Path., **29**:541–52, 1940.
8. LÖWENSTEIN, C. Über atypische Epithelwucherungen und Tumoren der Speicheldrüsen, besonders der Parotis. Frankfurt. Ztschr. f. Path., **4**:187–202, 1910.
9. MACCHIARULO, O., and BÜNGELER, W. Durch Teer erzeugte eigenartige Veränderungen der Parotisdrüse. Frankfurt. Ztschr. f. Path., **37**:211–21, 1929.
10. MCPEAK, E., and ARONS, W. L. Adenoacanthoma of the Esophagus: A Report of One Case with Consideration of the Tumor's Resemblance to So-called Salivary Gland Tumor. Arch. Path., **44**:385–90, 1947.
11. MCPEAK, E., and WARREN, S. Histologic Features of Carcinoma of the Cardio-Esophageal Junction and Cardia. Am. J. Path., **24**:971–91, 1948.
12. MEZA-CHÁVEZ, L. Sebaceous Glands in Normal and Neoplastic Parotid Glands. Am. J. Path., **25**:627–45, 1949.
13. RUSCH, H. P.; BAUMANN, C. A.; and MAISON, G. L. Production of Internal Tumors with Chemical Carcinogens. Arch. Path., **29**:8–19, 1940.
14. SCHAPER, A., and COHEN, C. Über zellproliferatorische Wachstumszentren und deren Beziehungen zur Regeneration und Geschwulstbildung. Arch. f. Entwcklngsmechn. d. Organ., **19**:348–445, 1905.
15. SKORPIL, F. Über das schleimbildende Epitheliom der Speicheldrüsen. Virchows Arch. f. path. Anat., **305**:661–84, 1940.
16. STEINER, P. E. Comparative Pathology of Induced Tumors of the Salivary Glands. Arch. Path., **34**:613–24, 1942.
17. TUREEN, L. L., and SEELIG, M. G. Reticulum in Tumors. Arch. Path., **15**:498–508, 1933.
18. WILLIS, R. A. Pathology of Tumours, pp. 140–41. St. Louis: C. V. Mosby Co., 1948.

Effect of Certain Folic Acid Antagonists on Transplanted Myeloid and Lymphoid Leukemias of the F Strain of Mice*

ARTHUR KIRSCHBAUM, PH.D., M.D., NANCY C. GEISSE, M.S.,† SISTER TERESITA JUDD, PH.D.,† AND LEO M. MEYER, M.D.

(From the Department of Anatomy, University of Minnesota Medical School, Minneapolis 14, Minn., and Department of Therapeutics, New York University College of Medicine, New York, N.Y.)

Transplanted mouse leukemia is being utilized as a test object for agents now being used in clinical treatment and as a screen for compounds which might conceivably be employed in the therapy of human leukemia (2, 6, 7). The objective of the present study was to test the influence of certain folic acid antagonists on the course and development of transplanted leukemia which originated in the F strain (9). Aminopterin and amethopterin, agents tested in this investigation, have been reported to exert significant therapeutic effects on acute leukemia of children and to delay the development of certain transfer lines of mouse leukemia (2–5, 10, 12, 15).

MATERIALS AND METHODS

Myeloid lines 15, 686, X, and 765 and lymphoid lines 876, 926, 794, and 100 are transfer lines of leukemia that were used in these experiments. The spontaneous cases from which they were established arose in the F strain. Lymphoid and myeloid leukemias of this strain of mice have been described (9).

Previous investigations had demonstrated that myeloid transfer lines of the F strain are influenced in their development to a greater degree than are lymphoid lines by either ethyl urethan or potassium arsenite (8). Inhibiting effects were obtained, however, only when treatment was begun within a few days after intraperitoneal inoculation of leukemic cells to effect transfer (7).

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The method of testing the folic acid antagonists[1] was the following: Leukemic cells of the respective transfer lines were inoculated into F_1 hybrid mice which were the offspring of an F strain male and a female of one of three foreign strains (CBA, Bagg albino, Strong A). Mice weighed from 14 to 20 gm. at the start of the experiments. Animals were given maximum tolerable doses of the materials tested; these doses were in the clinical dose range and were comparable to those used by other investigators using transplanted mouse leukemia. The drugs were dissolved in distilled water and given by intraperitoneal injection. The daily doses of the compounds used in this study are given in Table 1. Treatment was begun 24 hours after transfer; in certain experiments, where indicated, treatment was instituted at regulated intervals after transfer to compare the effects of the drug on the disease in different stages of development.

The following groups of controls were used. One set of mice was untreated, merely receiving the inoculum of leukemic cells suspended in isotonic saline. A second set was treated with potassium arsenite[2] and a third with ethyl urethan,[3] so that the effect of each folic acid antagonist was compared with that of each of the other drugs.

The number of leukemic cells inoculated for transfer was 1,000,000 in each mouse. The spleen of the leukemic donor was minced in several cc. of isotonic saline; the mince was filtered through several layers of cheesecloth. Counts were done on the resulting suspension of cells, with a standard white blood cell pipette and hemocytometer, and dilutions were made so that the number of cells injected intraperitoneally into each recipient mouse was contained in 0.1 cc. of isotonic saline. Mice in

[1] Supplied by Lederle Laboratories.

[2] Potassium arsenite, Merck.

[3] Urethan was obtained through the courtesy of Dr. L. E. Josselyn of Abbott Laboratories.

the test and control groups for each experiment were of similar genetic constitution, age, and weight. Single experiments involved five to ten test animals on each compound with a similar number of untreated controls. All mice in the treated groups that died prior to the death of the first control mouse were excluded from consideration. The figures in the tables represent the accumulated data of the individual experiments for each transfer line.

Animals were considered "positive" for leukemia if at autopsy the spleen was greatly enlarged, with at least a moderate increase in the size of liver and lymph nodes. Mice are listed as "negative" if they survived beyond 100 days after transfer and were negative at that time.

EXPERIMENTAL RESULTS

The following results were obtained when treatment with chemical agents was begun 24 hours (unless otherwise specified) following transplantation of leukemia by intraperitoneal inoculation of 1,000,000 leukemic cells.

Myeloid leukemias.—Potassium arsenite and ethyl urethan were more effective than any of the folic acid antagonists in delaying the development of leukemia in myeloid line 15. Twenty-five out of 37 mice treated with potassium arsenite did not develop leukemia. Although relatively ineffective, as compared to potassium arsenite and ethyl urethan, aminopterin and amethopterin increased survival time more than the other folic acid antagonists used (see Table 2).

In myeloid line 686, survival of mice treated with folic acid antagonists did not exceed that of untreated controls, whereas length of life after transfer was significantly increased in animals receiving either potassium arsenite or ethyl urethan. Eleven of 21 mice treated with potassium arsenite did not develop leukemia (see Table 3).

TABLE 1

COMPOUNDS TESTED FOR "ANTI-LEUKEMIC" EFFECT AND DAILY DOSE

Compound	Dose per day
Potassium arsenite (1 per cent in Fowler's solution)	0.1 mg.
Urethan (ethyl carbamate)	0.3 mg/gBW*
Aminopterin (4-amino-pteroyl-glutamic acid)	0.01 mg.
Amethopterin (4-amino-N-methylfolic acid)	0.05 mg.
Aninopterin (4-amino-9-methyl-pteroyl-glutamic acid)	0.01 mg.
Bremfol (x-methylfolic acid)	0.05 mg.
An-fol-R (pteroyl-DL aspartic acid)	0.04 mg/gBW
Met-fol-B (methol-pteroic acid)	0.04 mg/gBW
Amino-an-fol (4-amino-pteroyl-aspartic acid)	0.1 mg.
Amino-alan-fol (4-amino-pteroyl-alanine)	0.1 mg.
Amino-treo-fol (4-amino-pteroyl-threonine)	0.1 mg.
Teropterin (pteroyl-triglutamic acid)	0.04 mg-gBW
Folic acid (pteroyl-glutamic acid)	0.1 mg.

* Milligrams per gram body weight.

TABLE 2

EFFECT OF POTASSIUM ARSENITE, URETHAN, AND FOLIC ACID ANTAGONISTS ON SURVIVAL TIME IN MYELOID LINE 15, TRANSFER GENERATIONS 13 TO 33
(Treatment begun 24 hours after transfer of leukemia)

Number of mice	Compound	Survival in days		Mice surviving more than 37 days*	No. negative
		Range	Average		
99	Control	16–37	23	0	1
37	Potassium arsenite	29–100†	85†	33	25
27	Ethyl urethan	26–78	48	20	0
	Folic acid antagonists				
18	aminopterin	16–50	34	7	0
23	amethopterin	21–61	30	3	0
22	aninopterin	19–33	26	0	0
8	bremfol	19–31	24	0	0
9	an-fol-R	21–40	28	1	0
6	met-fol-B	18–31	25	0	0
6	amino-an-fol	21–27	25	0	0
6	folic acid	17–29	23	0	0
12	teropterin	24–38	28	1	0

* Longest control survival, 37 days.

† In computing averages, mice surviving 100 days or more were considered as surviving 100 days.

Folic acid antagonists were essentially without effect on myeloid line X. Survival was increased, however, when mice were treated with either potassium arsenite or ethyl urethan. The development of leukemia was inhibited in five of twenty cases treated with potassium arsenite, and in two of thirteen treated wth ethyl urethan (see Table 4).

The only folic acid antagonist which delayed the development of myeloid line 765 was aminopterin, but it was no more effective than ethyl urethan. Potassium arsenite was more effective than either, the development of the disease being inhibited in six of eleven mice, and all treated mice surviving longer than any untreated control (see Table 5).

TABLE 3

EFFECT OF POTASSIUM ARSENITE, URETHAN, AND FOLIC ACID ANTAGONISTS ON SURVIVAL TIME IN MYELOID LINE 686, TRANSFER GENERATIONS 7 TO 26
(Treatment begun 24 hours after transfer of leukemia)

Number of mice	Compound	Survival in days		Mice surviving more than 44 days*	No. negative
		Range	Average		
53	Control	20–44	27	0	0
21	Potassium arsenite	34–100†	75†	16	11
12	Ethyl urethan	19–87	43	5	0
	Folic acid antagonists				
5	aminopterin	25–100†	45†	1	1
10	amethopterin	22–31	25	0	0
3	aninopterin	22–26	24	0	0
3	amino-alan-fol	22–26	27	0	0
3	amino-treo-fol	21–31	25	0	0

* Longest control survival, 44 days.

† In computing averages, mice surviving 100 days or more were considered as surviving 100 days. The single negative mouse on aminopterin increases the "average survival."

TABLE 4

EFFECT OF POTASSIUM ARSENITE, URETHAN, AND FOLIC ACID ANTAGONISTS ON SURVIVAL TIME IN MYELOID LINE X, TRANSFER GENERATIONS 6 TO 14
(Treatment begun 24 hours after transfer of leukemia)

Number of mice	Compound	Survival in days		Mice surviving more than 36 days*	No. negative
		Range	Average		
25	Control	14–36	25	0	0
20	Potassium arsenite	23–100†	65†	19	5
13	Ethyl urethan	34–100†	61†	12	2
	Folic acid antagonists				
12	aminopterin	22–46	27	2	0
11	amethopterin	22–32	28	0	0
4	aninopterin	18–44	27	1	0

* Longest control survival, 36 days.

† In computing averages mice surviving 100 days or more were considered as surviving 100 days.

TABLE 5

EFFECT OF POTASSIUM ARSENITE, URETHAN, AND FOLIC ACID ANTAGONISTS ON SURVIVAL TIME IN MYELOID LINE 765, TRANSFER GENERATIONS 3 TO 8
(Treatment begun 24 hours after transfer of leukemia)

Number of mice	Compound	Survival in days		Mice surviving more than 29 days*	No. negative
		Range	Average		
18	Control	16–29	23	0	0
11	Potassium arsenite	35–100†	78†	11	6
11	Ethyl urethan	31–54	39	11	0
	Folic acid antagonists				
5	aminopterin	30–53	38	5	0
10	amethopterin	22–29	27	0	0
7	aninopterin	20–37	27	2	0
3	amino-alan-fol	24–27	26	0	0
3	amino-treo-fol	23–27	25	0	0

* Longest control survival, 29 days.

† In computing averages, mice surviving 100 days or more were considered as surviving 100 days.

Lymphoid leukemias.—If treatment of lymphoid line 876 was begun 24 hours after transfer, amethopterin was more effective than either potassium arsenite or urethan. When treatment was delayed until 5 or 10 days after transfer, then the effectiveness of amethopterin was reduced. When the mice were leukemic at the time transfer was instituted, that is, 10 days after transfer, amethopterin was actually without effect (see Table 6).

In transfer generations 2 and 3 of lymphoid line 876, when the average control survival was 36 days (average of 12 days in generations 16 through 24), all the drugs tested had an appreciably greater effect than in the later transfer generations. When treatment was begun 24 hours after transfer, all twelve mice given potassium arsenite survived longer than any control, and five mice did not develop leukemia. Seven of ten treated with ethyl urethan lived beyond any control, one remaining free of disease. Of nineteen treated with amethopterin, all survived beyond controls; seven did not develop leukemia.

For lymphoid transfer line 926, when treatment was begun 24 hours after transfer, amethopterin exerted a greater effect in delaying the development of transplanted leukemia than either potassium arsenite or ethyl urethan. The effect of amethopterin was reduced when treatment was begun 8 days after transfer. Amethopterin was the most effective of the folic acid antagonists used. In early (T_3) as well as later (T_{38}, T_{42}, T_{46}) transfer generations amethopterin was the most effective agent (see Table 7).

As in lymphoid lines 876 and 926, potassium arsenite and ethyl urethan were ineffective on lymphoid line 794 (contrasted with the effect on the myeloid lines) in appreciably increasing survival time, although treated mice did live slightly longer than controls. Amethopterin again delayed the development of leukemia to a greater extent than either of the preceding agents (see Table 8).

Although line 100 is a lymphoid transfer line, potassium arsenite and ethyl urethan exerted an inhibiting effect which was greater than that demonstrated by the folic acid antagonists (see Table 9). In the other three lymphoid lines the folic acid antagonists were effective, whereas in line 100 none appeared to exert any significant influence on survival time.

DISCUSSION

Different transfer lines of leukemia of the F strain exhibited individual responses to chemical agents. Response of lines could be correlated usually with their lymphoid or myeloid nature. Potassium arsenite either delayed or completely inhibited the development of the four myeloid lines tested when treatment was begun 24 hours after transfer. But the same drug was relatively ineffective on three of four lymphoid lines. Compared to potassium arsenite, the folic acid antagonists had relatively little effect on increasing survival time in the myeloid transfer lines. On the other hand, amethopterin, a folic acid antagonist, was more effective in three of the four lymphoid lines tested. This inhibitory action was apparent, however, only when treatment was begun within a few days after transfer effected by intraperitoneal inoculation of leukemic cells. If administration of the chemical was begun as much as 8 days after transplantation (average survival in controls 12 days), then survival did not significantly exceed that of untreated controls. When tested under optimum conditions, that is, beginning treatment 24 hours after transfer, in a responsive line of leukemia, aminopterin and amethopterin exerted more in-

TABLE 6

EFFECT OF POTASSIUM ARSENITE, URETHAN, AND A FOLIC ACID ANTAGONIST, AMETHOPTERIN, ON SURVIVAL TIME IN LYMPHOID LINE 876, TRANSFER GENERATIONS 16, 17, AND 24

(Treatment begun 24 hours after transfer of leukemia, except where otherwise indicated)

Number of mice	Compound	Survival in days		Mice surviving more than 14 days*	No. negative
		Range	Average		
17	Control	10–14	12	0	0
10	Potassiumarsenite	15–22	17	6	1
5 (D5)†	"	14–23	16	1	0
5 (D10)‡	"	14–23	16	2	0
10	Ethyl urethan	12–28	18	8	1
14	Amethopterin	20–33	25	14	2
5 (D5)†	"	14–21	18	3	0
5 (D10)‡	"	14	14	0	0

* Longest control survival, 14 days.
† Treatment begun 5 days after transfer.
‡ Treatment begun 10 days after transfer.

TABLE 7

Effect of Potassium Arsenite, Urethan, and Folic Acid Antagonists on Survival Time in Lymphoid Line 926

(Treatment begun 24 hours after transfer except where otherwise indicated)

Number of mice	Compound	Survival in days		Mice surviving more than:	No. negative
		Range	Average		
	Transfer generations 38 and 46				
				15 days*	
15	Control	10–15	12	0	0
6	Potassium arsenite	16–18	17	2	0
10	Ethyl urethan	16–27	22	5	0
14	Amethopterin	20–39	32	14	2†
10 (D4)‡	"	21–34	28	10	0
9 (D8)§	"	15–21	18	3	0
	Transfer generation 42				
				18 days*	
5	Control	12–18	15	0	0
5	Amethopterin	27–49	37	5	0
5 (D4)‡	"	21–32	26	5	0
5	Bremfol	12–18	15	0	0
5	Amino-an-fol	18–26	23	4	0
5	An-fol-R	12–19	14	1	0
	Transfer generation 3				
				21 days*	
4	Control	14–21	19	0	0
4	Potassium arsenite	20–26	22	2	0
5	Ethyl urethan	20–27	24	4	0
4	Aminopterin	19–29	21	1	0
5	Amethopterin	30–41	37	5	0

* Longest control survivals, 15, 18, and 21 days.

† Two negative animals died 24 and 27 days after transfer.

‡ Treatment begun 4 days after transfer.

§ Treatment begun 8 days after transfer.

TABLE 8

Effect of Potassium Arsenite, Urethan, and a Folic Acid Antagonist, Amethopterin, on Survival Time in Lymphoid Line 794, Transfer Generation 3

(Treatment begun 24 hours after transfer of leukemia)

Number of mice	Compound	Survival in days		Mice surviving more than 17 days*	No. negative
		Range	Average		
4	Control	14–17	16	0	0
5	Potassiumarsenite	22–29	24	5	0
5	Ethyl urethan	21–29	24	5	0
5	Amethopterin	18–43	31	5	0

* Longest control survival, 17 days.

TABLE 9

Effect of Potassium Arsenite, Urethan, and Folic Acid Antagonists on Survival Time in Lymphoid Line 100, Transfer Generations 3 to 7

(Treatment begun 24 hours after transfer of leukemia)

Number of mice	Compound	Survival in days		Mice surviving more than 35 days*	No. negative
		Range	Average		
24	Control	21–92	35	7	0
11	Potassium arsenite	35–100†	72†	11	4
10	Ethyl urethan	46–91	69	10	0
	Folic acid antagonists				
8	aminopterin	25–46	34	4	0
2	amethopterin	33–45	39	1	0
3	amino-an-fol	28–36	32	1	0
3	an-fol-R	26–36	30	1	0
3	met-fol-B	29–36	33	1	0

* Average control survival, 35 days.

† In computing averages, mice surviving 100 days or more were considered as surviving 100 days.

hibiting action than the other folic acid antagonists. In no instance, however, were these agents as effective for a lymphoid line as was potassium arsenite for all of four myeloid lines. Using "effective" doses of aminopterin and amethopterin resulted in the death of many test animals before they became leukemic, although the survivors maintained their body weight almost as well as controls.

The extent to which inhibition of the development of transplanted leukemia is a critical means of assaying the therapeutic efficiency of chemical agents is debatable. Only certain lines are susceptible to specific agents, and in these leukemias inhibitory action is demonstrable only during the period preceding generalized infiltration by leukemic cells. Although colchicine induced regression of locally established transplanted lymphosarcomata (1, 11), this drug was ineffective upon the widely disseminated lymphoid neoplasm (11).

Early transfer generations of certain lines of leukemia (876, 868) were "indiscriminately" sensitive to several agents, although later transfers were not significantly affected. Twelve out of fifteen mice of the second transfer generation of line 868 did not develop leukemia when treated with either ethyl urethan, potassium arsenite, aminopterin, or amethopterin. Sixteen of 49 in the second and third transfers of line 876 were negative, although all of 14 animals of generations 16 and 17 which were similarly treated developed leukemia.

The reason for inhibition by certain agents is not understood. Lengthening of the latent period, or complete inhibition, might result from destruction of leukemic cells which are very sensitive to the drug during the few days following their inoculation to effect transfer. The inhibiting effect is significant, since it is not associated with all drugs. Certain arsenicals and carbamates not far removed chemically from potassium arsenite (e.g., arsenic acid) and ethyl urethan (e.g., methyl carbamate) have proved to be relatively ineffectual.

Although line 15 was completely inhibited by potassium arsenite in a majority of the cases when treatment was begun 24 hours after transfer, certain animals developed leukemia. When cells from such leukemic mice were used for transfer the response to potassium arsenite was the one ordinarily observed, indicating that the cells upon first transplantation from a treated mouse were not resistant to the drug. These studies are being extended.

In treating continuously with the various agents it became apparent that survival periods for mice in any transfer line were usually similar after the spleen had become palpable, even though treatment was maintained. The effect of any agent was *to prevent or delay the development of the disease;* the course of the full-blown disease was not altered.

Other investigators have described the inhibition of certain transfer lines of leukemia by the administration of folic acid antagonists (2, 10). In their studies the inhibitory action was almost "line specific," certain transfer lines being refractory to "effective" agents. These studies were done on animals in which treatment was begun no more than 4 days after transplantation. When other neoplasms have been inhibited by this group of chemicals, treatment was begun shortly after transplantation (13, 14). Therapy probably interfered with the establishing of the tumor-host relationship which allows for proliferation of transplanted cells.

SUMMARY

Certain folic acid antagonists, including aminopterin and amethopterin, either failed to increase survival time or were less effective than potassium arsenite and ethyl urethan on four transfer lines of myeloid mouse leukemia of the F strain. The chemicals were administered daily in maximum tolerable doses. Treatment was begun 24 hours after transplantation effected by intraperitoneal inoculation of 1,000,000 leukemic cells.

In certain lymphoid transfer lines, amethopterin, a folic acid antagonist, increased survival time to a greater extent than either potassium arsenite or urethan. This inhibiting action was not demonstrable, however, when treatment was begun 8 days after transplantation, untreated controls surviving 10–14 days. Thus, certain agents which increase survival time in specific transplanted leukemias do not affect the course of the disease once the organs are generally infiltrated with leukemic cells. The effectiveness of specific agents is probably due to a delay in the establishment of the usual leukemic cell-host relation of successful transplants.

The inhibiting action of these specific agents on transplanted leukemia has some pharmacologic significance, since even closely related chemicals may be ineffective.

REFERENCES

1. BASS, A. D., and PROBERT, C. Response of a Transplantable Lymphosarcoma to Colchicine. Cancer Research, **10:** 420–22, 1950.
2. BURCHENAL, J. H.; BURCHENAL, J. R.; KUSHIDA, M. N.; JOHNSTON, S. F.; and WILLIAMS, B. S. Studies on the Chemotherapy of Leukemia. II. The Effect of 4-Amino-Pteroylglutamic Acid and 4-Amino-N^{10}-Methyl-Pteroylglutamic Acid on Transplanted Mouse Leukemia. Cancer, **2:** 113–20, 1949.

3. Damashek, W. The Use of Folic Acid Antagonists in the Treatment of Acute and Subacute Leukemia. Blood, **4**: 168–71, 1949.
4. Farber, S. Some Observations on the Effect of Folic Acid Antagonists on Acute Leukemia and other Forms of Incurable Cancer. Blood, **4**: 160–67, 1949.
5. Farber, S ; Diamond, L. K.; Mercer, R. D.; Sylvester, R. F., Jr.; and Wolff, J. A. Temporary Remission in Acute Leukemia in Children Produced by Folic Acid Antagonist, 4-Aminopteroylglutamic Acid (Aminopterin). New England J. Med., **238**: 787–93, 1948.
6. Flory, C. M.; Furth, J.; Saxton, J. A., Jr.; and Remer, L. Chemotherapeutic Studies on Transmitted Mouse Leukemia. Cancer Research, **3**: 729–43, 1943.
7. Geisse, N., and Kirchsbaum, A. Transplanted Mouse Leukemia as a Test Object for the Evaluation of Chemotherapeutic Agents. Cancer Research, **10**: 108–12, 1950.
8. Kirschbaum, A.; Judd, Sister T.; Lu, C. S.; Engstrom, R.; and Mixer, H. Effect of Urethane, X-Rays, Potassium Arsenite, Benzol, on Survival Time in Transplanted Mouse Leukemia. Proc. Soc. Exper. Biol. & Med., **68**: 377–80, 1948.
9. Kirschbaum, A., and Strong, L. C. Leukemia in the F Strain of Mice: Observations on Cytology, General Morphology, and Transmission. Am. J. Cancer, **37**: 400–413, 1939.
10. Law, L. W.; Dunn, T. B.; Boyle, P. J.; and Miller, J. H. Observations on the Effect of a Folic Acid Antagonist on Transplantable Lymphoid Leukemias in Mice. J. Nat. Cancer Inst., **10**: 179–92, 1949.
11. Lits, F. J.; Kirschbaum, A.; and Strong, L. C. Action of Colchicine on a Transplanted Malignant Lymphoid Neoplasm in Mice of the C3H Strain. Am. J. Cancer, **34**: 196–213, 1938.
12. Meyer, L. M.; Fink, H.; Sawitsky, A.; Rowen, M.; and Ritz, N. D. Aminopterin (a Folic Acid Antagonist) in the Treatment of Leukemia. Am. J. Clin. Path., **19**: 119–26, 1949.
13. Schoenbach, E. B.; Golden, A.; Goldberg, B.; and Ortega, L. G. The Effect of Folic Acid Derivatives on Sarcoma 180. Cancer, **2**: 57–63, 1949.
14. Sugiura, K.; Moore, A. E.; and Stock, C. C. The Effect of Aminopterin on the Growth of Carcinoma, Sarcoma, and Melanoma in Animals. Cancer, **2**: 491–502, 1949.
15. Weir, D. R.; Welch, A. P.; and Heinle, R. W. Effect of an Antagonist of Pteroylglutamic Acid on the Leukemia of Ak Mice. Proc. Soc. Exper. Biol. & Med., **71**: 107–15, 1949.

The Effect of Protein Depletion on the Host Response to Transplantable Rat Tumor Walker 256

JOHN W. GREEN, JR., M.D., EARL P. BENDITT, M.D., AND ELEANOR M. HUMPHREYS, M.D.

(From the Department of Pathology, University of Chicago, Chicago 37, Ill.)

INTRODUCTION

The nutritional state of the host has been established as an important factor in the growth of transplantable rat and mouse tumors (7, 11) and in the induction and spontaneous occurrence of several types of mouse tumors (7, 8, 12, 13, 16). It has been shown that transplantable tumors in the mouse grow at the expense of body nitrogen if insufficient dietary nutrition is available (14, 15). In addition to the influence of the host on the tumor, several changes in the host have been described that apparently result from the presence of a transplantable or induced tumor. The livers of tumor-bearing rats have been shown to increase in weight (6, 18) and water content (6, 9, 10). Increased blood water content has also been described (9). Transplantable granulosa-cell tumors in the mouse may cause an increase in blood volume (4). The present experiments were undertaken to study the liver water and blood volume changes simultaneously in the tumor-bearing rat, and to determine if the host response to the tumor could be modified by protein depletion.

MATERIALS AND METHODS

Young male rats of the Sprague-Dawley strain ranging in weight from 200 to 225 gm. were used in these experiments. Protein depletion was accomplished by the method of Cannon and his group (17). This consisted of feeding a diet adequate in all nutritional factors but protein for 8–12 weeks, until the animals had lost 25–35 per cent of their pre-depletion body weight. The depletion diet[1] contained about 2 per cent protein as carrots, brewer's yeast, and liver powder concentrate, and it was adequate in vitamins, minerals and calories. Nondepleted control rats of the same age were maintained on a high protein casein diet[2] or Purina Laboratory Chow until the experiments were begun. During the experimental period, synthetic diets were used. The depleted animals were fed a low protein diet that contained less than 1 per cent of protein.[3] The nondepleted animals were fed a 22 per cent casein diet.[4] These diets were isocaloric. They were fed at a level of 15 gm/rat/day, but the depleted animals did not consume all their daily ration. Because of the difference in consumption between depleted and nondepleted animals, their caloric intake was not directly comparable. The presence of the tumor in both depleted and nondepleted animals did not have any pronounced effect on diet consumption compared with their respective controls.

The tumor used in these experiments was the transplantable Walker tumor 256, which was inoculated in 0.2-cc. amounts of a sterile saline suspension into the skin of the back. The details of the technic are given elsewhere (5). Beginning on the fourth day after transplantation and extending until the fourteenth day, the tumors were measured in three dimensions by calipers to obtain an estimate of the volume. The estimates of tumor volume were plotted on log-log graph paper to obtain straight line growth curves. In this way the slopes of the curves of different experiments could easily be compared. The experimental period

[1] Composition per 100 grams of diet: carrots, 30 gm.; ruffex, 5 gm.; lard, 4 gm.; cornstarch, 44 gm.; O and M salt mix (modified), 4 gm.; brewer's yeast, 2 gm.; liver concentrate, 1 gm.; water, 10 cc.; riboflavin, 500 γ; pyrodixine HCl, 200 γ; calcium pantothenate, 200 γ; choline chloride, 100 mg.; vitamin A, 200 USP units; vitamin D, 29 USP units.

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[2] Composition per 100 grams: identical with the depletion diet, except that 22 gm. of vitamin test casein replaced 22 gm. of cornstarch.

[3] Composition per 100 grams: dextrin, 71 gm.; ruffex, 5 gm.; O and M salt mix (modified), 4 gm.; corn oil, 4 gm.; water, 13.3 cc.; choline chloride, 200 mg.; thiamin, 540 γ; riboflavin, 1,080 γ; niacin, 2,840 γ; pyridoxine HCl, 640 γ; calcium pantothenate, 1,750 γ; vitamin A, 400 USP units; vitamin D, 60 USP units.

[4] Composition per 100 grams: dextrin, 49.6 gm.; ruffex 5 gm.; O and M salt mix (modified), 4 gm.; corn oil, 4 gm.; water, 11.17 cc.; casein (vitamin test), 23.53 gm. Vitamins are identical with those listed in footnote 3.

lasted for 14 days after the inoculation of the tumors.

On the day of inoculation of the tumors, depleted and nondepleted animals of the same age and nutritional state as the experimental animals were sacrificed as base-line controls after serum protein concentration and hemoglobin concentration had been obtained. On the fourteenth day of tumor growth, the serum protein concentration, blood volumes, and hemoglobin concentration were determined in both tumor-bearing and nontumor-bearing animals which then were sacrificed by exsanguination after light ether anesthesia. The tumors and liver were excised and weighed, the gastro-intestinal tract was excised and cleaned of retained food and fecal material. The carcass weight was obtained by including the cleaned gastro-intestinal tract with the carcass and remaining viscera. Duplicate samples of liver and tumor were analyzed for water, fat, water-soluble fraction content, and protein residue by a rapid gravimetric method.[5] The water content was determined by drying to a constant weight at 70° C., the fat content by hot extraction by commercial absolute alcohol followed by cold ether extraction. The water-soluble fraction was obtained by water extraction at 70° C. after the removal of alcohol and ether-soluble material. The residual dried sample consisted of "protein residue." The gravimetric method protein, as determined by the Kjehldahl method, using the factor $N \times 6.25$, ranged between 90 and 95 per cent of the "protein residue." In the data presented, the results of the liver analysis are expressed in per cent, corrected for the fat-free weight of the liver. Previous experiments have shown that fat is the most variable component, and comparability of values of nonlipid constituents is attained when expressed on a fat-free basis.

Carcass analysis was performed by a similar procedure. The protein residue values include the ash content of the carcasses and are expressed as fat-free dry weight.

Serum protein concentration was determined by the falling drop method (1) and hemoglobin concentration with the Dick-Stevens hemoglobinometer. The total blood volumes and plasma volumes were determined by the Benditt *et al.* modification of the Evans blue dye technic for rats (2).

The data from two separate experiments were pooled and combined in the final evaluation of results, since both procedures and results were comparable in each separate experiment.[6]

[5] E. M. Humphreys, to be published.

[6] L. J. Savage, personal communication.

EXPERIMENTS AND RESULTS

The results are summarized in Table 1.

Effect of protein depletion on the growth of Walker 256 tumor.—Protein depletion of the host had a profound effect on the growth of the tumor. The maximum tumor weight obtained in the depleted animals was 7.04 gm., compared with a maximum of 31.72 gm. in the nondepleted group. The state of the host apparently did not affect the rate of growth of the tumors but only prolonged the latent period.[7] Figure 1 is a graph of the separate growth curves defined by plotting the estimated volumes of the tumors against time. It can be seen that, although the tumors from depleted animals were always smaller, the slope of the points was approximately the same.

Comparison of the chemical analyses of the tumors from depleted and nondepleted animals revealed a significant lowering of protein content of the depleted tumors compared to those of the nondepleted animals. Tumors from depleted animals showed consistently less necrosis in their center and hence had less extra-vascular leakage of blood and plasma.

Effect of protein depletion on changes in liver composition associated with Walker 256 tumor.—The presence of the tumor in nondepleted rats was associated with a gross increase in liver wet weight compared with nontumor-bearing animals. The mean weight of the livers from tumor-bearing rats was 8.27 gm., compared with the nontumor-bearing control liver weight of 6.53 gm. This increase in liver wet weight was not observed in the depleted tumor-bearing animals. These did not differ significantly from the depleted controls, as is shown in Table 1.

Associated with the increased wet weight of the livers of the nondepleted tumor-bearing animals, there was an increased water content; this was 76.03 per cent compared to 74.28 per cent for the controls. The livers of all depleted animals showed a higher water content than those of the nondepleted group; however, those from tumor-bearing animals also had a greater water content than those from the controls—78.91 per cent, compared to 77.3 per cent.

The liver protein residue was reduced in the presence of the tumor in both depleted and nondepleted animals. The values were 20.38 per cent for the nondepleted tumor-bearing animals, compared to 22.53 per cent for the controls. Depleted tumor-bearing and control protein residues were 17.23 per cent and 18.94 per cent, respectively.

The water-soluble fraction of the liver was

[7] Latent period as used here means the time after inoculation of the tumor until it is palpable.

TABLE 1

Summary of Data on the Effect of Protein Depletion on Host Physiology in the Tumor-bearing Rat

	Number animals	Wt.* 1st day exp.	Wt. 14th day exp.	Net wt.† change		Carcass‡ Wet wt.	Water	Fat	Fat-free dry wt.	Blood Plasma vol. (cc.)	Red cell vol. (cc.)	Serum prot. concentration (gm. per cent)	TCSP§ (mg.)	Hemoglobin (gm. per cent)
Depleted:														
Base line	9	146.41			Mean	*132.61*	*85.60*	*12.70*	*34.31*					
					S.E.	4.32	2.80	0.72	0.94					
					Mean	*125.85*	*82.17*	*10.02*	*33.66*	*6.68*	*2.99*	*4.45*	*297.25*	*10.68*
No tumor	8	151.13	138.0	−13.13	S.E.	4.21	1.70	1.61	0.79	0.40	0.21	0.16	21.49	1.14
					Mean	*117.06*	*78.88*	*7.51*	*30.68*	*6.77*	*3.09*	*3.64*	*244.10*	*10.67*
With tumor	20	147.20	136.75	−14.92	S.E.	2.11	1.30	0.52	0.44	0.15	0.09	0.058	4.83	0.37
P value‖						0.05	N.S.	N.S.	<0.01 >0.001	N.S.	N.S.	<0.001	<0.01 >0.001	N.S.
Nondepleted:														
Base line	10	228.68			Mean	*206.12*	*139.33*	*19.67*	*47.12*					
					S.E.	2.63	2.46	1.27	0.47					
					Mean	*211.81*	*139.21*	*21.01*	*51.09*	*9.11*	*7.57*	*6.36*	*579.50*	*16.58*
No tumor	10	232.30	238.0	+ 5.70	S.E.	4.18	2.28	1.61	0.70	0.25	0.25	0.077	15.26	0.65
					Mean	*206.55*	*138.57*	*17.41*	*50.58*	*10.86*	*6.06*	*5.11*	*551.35*	*12.08*
With tumor	20	231.85	255.35	+ 4.04	S.E.	2.62	1.74	1.18	1.27	0.22	0.24	0.10	9.38	0.52
P value‖						N.S.#	N.S.	N.S.	N.S.	<0.001	<0.001	<0.001	N.S.	<0.001

	Number animals	Wt. 1st day exp.	Wt. 14th day exp.	Net wt. change		Liver analysis Wet wt.	Per cent water	Per cent water-sol. solids	Per cent protein residue	Per cent fat	Tumor analysis Excised wt.	Per cent protein residue	Per cent water	Per cent fat
Depleted:														
Base line	9	146.41			Mean	*4.37*	*77.42*	*4.21*	*18.92*	*8.16*				
					S.E.	0.22	0.35	0.14	0.31	0.73				
					Mean	*4.30*	*77.30*	*3.76*	*18.94*	*6.83*				
No tumor	8	151.13	138.0	−13.13	S.E.	0.20	0.24	0.16	0.32	0.50				
					Mean	*4.15*	*78.91*	*3.85*	*17.23*	*6.79*	*4.47*	*8.94*	*84.63*	*2.71*
With tumor	20	147.20	136.75	−14.92	S.E.	0.089	0.27	0.091	0.22	0.22	0.32	0.16	0.43	0.087
P value‖						N.S.	<0.001	N.S.	<0.001	N.S.				
Nondepleted:														
Base line	10	228.68			Mean	*7.60*	*74.05*	*2.90*	*23.05*	*5.83*				
					S.E.	0.61	0.15	0.14	0.15	0.003				
					Mean	*6.53*	*74.28*	*3.17*	*22.53*	*5.77*				
No tumor	10	232.30	238.0	+ 5.70	S.E.	0.083	0.082	0.08	0.15	0.15				
					Mean	*8.27*	*76.03*	*3.57*	*20.38*	*6.43*	*19.47*	*9.54*	*84.20*	*2.62*
With tumor	20	231.85	255.35	+ 4.04	S.E.	0.17	0.51	0.08	0.17	0.098	1.35	0.17	0.17	0.086
P value‖						<0.001	<0.01 >0.001	<0.01 >0.001	<0.001	<0.001	<0.001	<0.02 >0.01	N.S.	N.S.

* All weights expressed in grams.

† Net weight of tumor-bearing animals equals total weight minus tumor weight.

‡ All carcass data expressed in grams.

§ Total circulating serum protein.

‖ Significance of difference of the means of tumor-bearing and nontumor-bearing rats.

\# Not statistically significant.

slightly increased in the nondepleted tumor-bearing animals only. No difference was noted in the depleted animals. Analysis for fat revealed a slight increase in the tumor-bearing nondepleted animals. Fat levels were similar in the livers of depleted animals with and without tumors.

Effects of the Walker tumor on blood volume and plasma protein.—In the nondepleted tumor-bearing rats, there was a consistent increase in plasma volume. The mean plasma volume was 10.86 cc.,

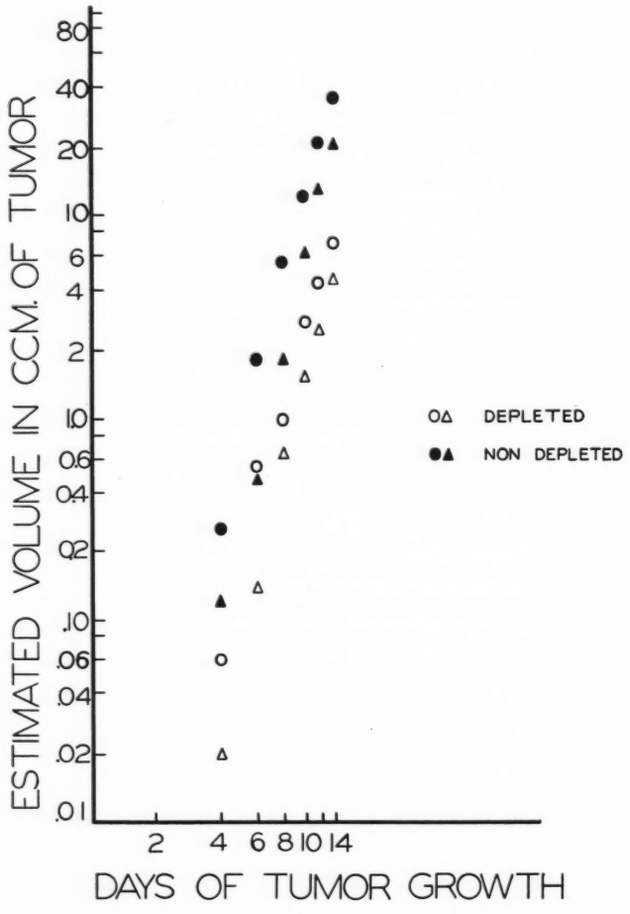

FIG. 1.—Plot of tumor growth (log-log scale). The triangles and circles represent various experiments. It can be seen that all points fall along approximately straight and parallel lines.

as compared to 9.11 cc. for the controls. The red cell volume and hemoglobin concentrations were diminished. Associated with the increased plasma volume, there was a decrease in serum protein concentration, although the total circulating serum protein was not affected. In the protein-depleted rat the presence of a tumor had no effect on the plasma volume response, and there was no change in the red cell volume or hemoglobin concentration. However, the serum protein concentration and total circulating serum protein were diminished in the tumor-bearing depleted animals.

Effect of protein depletion on the carcass of tumor-bearing rats.—Presence of the tumor did not consistently alter the chemical content of the carcass of either the depleted or nondepleted animals, except for a diminution of carcass protein residue and wet weight in the depleted group.

DISCUSSION

The protein-depleted state has a profound effect on many physiological activities. Since a tumor-bearing animal on a low protein diet has a strictly limited exogenous source of protein and is in negative nitrogen balance, it must supply the requirements of the tumor by depleting its own tissues. It is not surprising that the maximum size of the tumors is much smaller than those in nondepleted hosts. With the smaller tumors in the depleted animals one might expect a less profound effect of the tumor upon the host. This is seen in these experiments by the failure of the liver to increase in weight in the depleted tumor-bearing animals and by the failure of the tumors to elicit an increased plasma volume and hemodilution. It is possible that our methods failed to detect minor changes in plasma volume.

The effect produced on the host may be a simple function of the mass of the tumor. Yeakel (18) noted that the weight of the livers of tumor-bearing rats was a function of tumor weight and body length and that the effect was greater in females than in males. McEwen and Haven (6) observed a significant increase in the percentage of water in the livers of rats bearing the Walker 256 tumor. A critical tumor weight of 10 gm. or more was thought to be necessary before liver water content was increased. Schlottman and Rubenow (9) found a higher percentage of water in the livers of rats bearing the Jensen sarcoma and the Flexner carcinoma. In their experiments the increased liver water was associated with an increased water content of the blood, while the wet weight of the livers was not increased. The present study has confirmed the increase in liver water of tumor-bearing rats, both depleted and nondepleted. Our experiments have shown that the water content of the rat liver was increased in nondepleted rats with large tumors and in depleted animals whose mean tumor weight was less than 5 gm.

Furth and Sobel (4) reported that greatly increased blood volume occured in mice bearing some types of granulosa-cell tumors. They found an absolute increase in both plasma and red cell volume and suggested that the hypervolemia might be related to a substance elaborated

by the granulosa cells of the tumors. Mice bearing mammary tumors of the same size or leukemia did not show the increase in blood volume. In the present study increased plasma volume with decreased red cell volume was observed only in the nondepleted tumor-bearing rats. The determining factor here may be tumor size, since the tumors in the depleted animals were much smaller. The increased plasma volume does not appear to be a compensatory mechanism for the lowering of serum proteins, but rather a phenomenon of hemodilution, since the total circulating serum protein was not significantly changed.

The lowering of the liver protein residue in both depleted and nondepleted tumor-bearing animals is to be expected, since the liver is a ready source of immediately available protein. In the depleted animals, the carcass appears to supply the majority of the protein for tumor protein synthesis. Obviously, the high protein intake provided a primary source in the nondepleted animals.

There is a significant difference in protein residue of the tumors of the depleted and nondepleted hosts. The tumors from depleted animals appear to contain less protein (Table 1). The cause of this difference is not apparent, but it seems likely that more necrosis and hence more leakage of blood into large tumors would give them an apparently higher total protein content.

Statistical correlation of the important variables in these experiments clarifies these differences (3). The pooled correlation for all depleted and nondepleted animals of the liver weight with the plasma volume, tumor weight, or carcass weight was approximately the same: 0.562, 0.565, and 0.548, respectively. The plasma volume was highly correlated with the tumor weight (0.669) and showed essentially no correlation with the carcass weight (0.297), nor was the tumor correlated with the carcass weight (-0.0349). By partial correlation it was found that the correlation of plasma volume and weight of the tumor increased to 0.710 at constant carcass weight. This analysis indicates that the plasma volume and the liver water changes are closely related to the presence of the tumor. The increased plasma volume in the animals bearing large tumors may simply be an attempt to keep full the rapidly expanding vascular bed of the tumor, and the increment may approximate the volume of the vascular bed of the tumor. Other factors which might cause a rise in plasma volume by hormonal mechanisms cannot be excluded. About 75 per cent of the difference in liver weight between nondepleted tumor-bearing and control rats is due to the increased water content of the livers of the tumor-bearing rats.

SUMMARY

The effects of protein depletion on tumor growth and the response of the host to the presence of transplantable Walker 256 tumor were studied in rats. Protein depletion had a profound effect on the growth of the tumor in a 14-day period. Tumors in rats depleted of protein were less than one-fourth as large as those in nondepleted rats. The primary effect seemed to be on the latent period of the tumors of the depleted animals, since the relative rates of growth of tumors in depleted and nondepleted rats were comparable. In the nondepleted tumor-bearing animals, there was an increased plasma volume, liver wet weight, and increased water content of the liver. The presence of the tumor in the depleted host did not cause an increase in plasma volume or liver wet weight, but it was associated with an increased water content of the liver. Total circulating serum protein was not altered in conjunction with the increased plasma volume of the nondepleted rats. The concentration of liver protein was decreased in both the nondepleted and depleted tumor-bearing animals.

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REFERENCES

1. BARBOUR, H. G., and HAMILTON, W. F. The Falling Drop Method for Determining Specific Gravity. J. Biol. Chem., **69**:625–40, 1926.
2. BENDITT, E. P.; STRAUBE, R. L.; and HUMPHREYS, E. M. The Determination of Total Circulating Serum Proteins and Erythrocyte Volumes in Normal and Protein-depleted Rats. Proc. Soc. Exper. Biol. & Med., **62**:189–92, 1946.
3. FISHER, R. A. Statistical Methods for Research Workers, pp. 175–97. 11th ed. Edinburgh: Oliver & Boyd, Ltd., 1950.
4. FURTH, J., and SOBEL, H. Hypervolemia Secondary to Grafted Granulosa-Cell Tumor. J. Nat. Cancer Inst., **7**: 103–13, 1946.
5. GREEN, J. W., and LUSHBAUGH, C. C. Histopathologic Study of the Mode of Inhibition of Cellular Proliferation by Urethane: Effect of Urethane on Walker Rat Carcinoma 256. Cancer Research, **9**:199–209, 1949.
6. MCEWEN, H. D., and HAVEN, F. L. The Effect of Carcinosarcoma 256 on the Water Content of the Liver. Cancer Research, **1**:148–50, 1941.
7. Rous, P. The Influence of Diet on Transplanted and Spontaneous Mouse Tumors. J. Exper. Med., **20**:433–51, 1914.
8. SAXTON, J. A.; BOON, M. C.; and FURTH, J. Observations on the Inhibition of Development of Spontaneous Leukemia in Mice by Underfeeding. Cancer Research, **4**:401–9, 1944.
9. SCHLOTTMAN, H., and RUBENOW, W. Über den Wassergehalt der Gewebe normaler und krebskranker Ratten. Ztschr. f. Krebsforsch., **36**:120–25, 1932.
10. SHERMAN, C. D.; MORTON, J. J.; and MIDER, G. B. Potential Sources of Tumor Nitrogen. Cancer Research, **10**: 374–78, 1950.

11. Sweet, J. E.; Corson-White, E. P.; and Saxon, G. J. The Relation of Diets and of Castration to the Transmissible Tumors of Rats and Mice. J. Biol. Chem., **15**:181–91, 1913.
12. Tannenbaum, A. Effects of Varying Caloric Intake upon Tumor Incidence and Tumor Growth. Ann. New York Acad. Sc., **49**:5–18, 1947.
13. ———. The Dependence of Tumor Formation on the Degree of Caloric Restriction. Cancer Research, **5**:609–15, 1945.
14. White, F. R. Source of Tumor Proteins. II. Nitrogen-Balance Studies of Tumor-bearing Mice Fed a Low-Nitrogen Diet. J. Nat. Cancer Inst., **5**:265–68, 1945.
15. White, F. R., and Belkin, M. Source of Tumor Proteins. I. Effect of a Low-Nitrogen Diet on the Establishment and Growth of a Transplanted Tumor. J. Nat. Cancer Inst., **5**:261–63, 1945.
16. White, F. R.; White, J.; Mider, G. B.; Kelley, M. G.; Heston, W. E.; and David, P. W. Effect of Caloric Restriction on Mammary-Tumor Formation in Strain C3H Mice and on the Response of Strain DBA to Painting with Methylcholanthrene. J. Nat. Cancer Inst., **5**:43–48, 1944.
17. Wissler, R. W.; Woolridge, R. L.; Steffee, C. H.; and Cannon, P. R. The Relationship of the Protein-Reserves to Antibody Production. II. The Influence of Protein Repletion upon the Production of Antibody in Hypoproteinemic Adult White Rats. J. Immunol., **52**: 267–79, 1946.
18. Yeakel, E. H. Increased Weight of the Liver in Wistar Albino Rats with Induced and Transplanted Tumors. Cancer Research, **8**:392–96, 1948.

Production of Neoplasms by Injection of Fractions of Mammalian Neoplasms*

JOSEPH STASNEY, M.D., A. CANTAROW, M.D., AND KARL E. PASCHKIS, M.D.†

(From the Division of Endocrine and Cancer Research, Jefferson Medical College, Philadelphia, Pa.)

The discovery that certain tumors may be induced in certain species by viruses led to attempts to transmit mammalian tumors by injection of cell-free preparations. Taylor and co-workers (7, 8) claimed success with such material, but others failed to corroborate their findings (2, 9). We are aware of no previous report of transmission of a mammalian neoplasm by injection of isolated cytoplasmic or nuclear components of tumor cells.[1]

MATERIALS AND METHODS

Two types of tumor were employed: (*a*) 2-acetaminofluorene-induced hepatomas in rats and (*b*) the Murphy rat lymphosarcoma.

AAF-induced Hepatomas

Hepatomas were induced in male Wistar rats by feeding 2-acetaminofluorene (3). Tumor tissue was obtained either from single large hepatomas or from several pooled smaller tumors.

Preparation of mitochondria fraction.—Five to 8 gm. of tissue was homogenized in a refrigerated Waring Blendor in a cold-room (2°–4° C.), under aseptic conditions, in either 0.85 per cent NaCl solution or 0.88 M sucrose solution (dilution 1:10). The material was fractionated in a refrigerated centrifuge by the differential centrifugation technic of Claude (4) as modified by Hogeboom *et al.* (6). Cell remnants, nuclei, and erythrocytes were removed by centrifuging the homogenate at 600 *g* for 10 minutes. This procedure was repeated twice, the sediment being discarded and the supernatants pooled.

The mitochondria fraction was isolated by centrifuging the pooled supernatant at 24,000 *g* for 20 minutes; the sediment was resuspended in NaCl or sucrose solution and recentrifuged at 24,000 *g* for 20 minutes.

Smears and sections of paraffin-imbedded preparations were made from every sample of this fraction. These were stained with Janus green B and Mallory's phosphotungstic acid-hematoxylin stain, respectively.

One hundred gm. of normal liver was fractionated in the same manner, the mitochondria fraction was suspended in 0.85 per cent NaCl solution containing a little methylene blue, and the entire sample was examined carefully in a blood-counting chamber.

Preparation of chromatin fraction.—Under aseptic conditions, 20–30 gm. of tissue was ground with an equal weight of beach sand for 5 minutes, with 100 cc. of buffered (pH 7.4) 0.9 per cent NaCl solution added progressively. The triturated mixture was passed through a double layer of gauze and transferred to centrifuge tubes. The subsequent procedure was that of differential centrifugation described by Claude and Potter (5).

The following experimental procedures were employed, under aseptic conditions:

1. Saline suspensions of intact tumor cells (0.2–0.3 ml.) were injected into the liver in 23 normal adult Wistar rats at laparotomy under ether anesthesia. The surface opening was sealed with Oxycel.

2. The mitochondria fraction was injected in the same manner, suspended in saline in 45, and in sucrose in 73, cases.

3. The chromatin fraction, suspended in 0.2–0.3 ml. saline, was injected in the same manner in fifteen animals.

4. Similar preparations of normal liver cells

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† From the Department of Pathology, the Department of Biochemistry, and the Departments of Physiology and Medicine, respectively.

[1] While this manuscript was in press, a publication by Hans Lettré (Die Naturwissenschaften, **14**:335–36, 1950) came to our attention, in which was reported the production of mouse ascites tumor by intraperitoneal injection of "plasma granules," purported to be mitochondria, and of the "granule-free" cells, independently of each other. No tumors followed injection of either material alone.

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were injected in the same manner in 23 animals.

5. Crystals of 2-acetaminofluorene, approximately 0.5 mg., were suspended in rat plasma, which was subsequently coagulated and implanted in the liver in twelve rats.

All animals were sacrificed after 12–98 days.

Murphy Rat Lymphosarcoma[2](10)

Actively growing tumors were obtained from Dr. Murphy and were continued by transplantation in our animal colony (Wistar-Carworth rats).

Three types of preparation of this tumor were employed:

1. Suspensions in 1-ml. 0.85 per cent NaCl solution of intact cells representing approximately 250 mg. of tumor tissue.

2. Suspensions in 1- or 2-ml. NaCl solution of the mitochondria fraction obtained from 20–40 gm. of tissue.

3. Suspensions in 1- or 2-ml. NaCl solution of the chromatin fraction obtained from 20–40 gm. of tissue.

Cell suspensions and chromatin fractions were prepared also from several of the tumors which developed after subcutaneous injection of the chromatin fraction of the original tumor.

These preparations (1 ml.) were injected subcutaneously (groin), and into the spleen, bone marrow, and thymus, the number of animals used in each case being indicated in Table 2. Intact cell suspensions were also injected into the liver in three animals (not indicated in the table).

The following procedures were employed in an attempt to establish the "purity" of the chromatin fraction—especially to eliminate, in so far as was possible, the possibility of contamination with intact cells:

1. Smears were made of a portion of each injected chromatin fraction. These were stained with toluidine-blue, Wright's stain, and by the Feulgen technic.

2. Suspensions were made, in 1-ml. NaCl solution, of intact tumor cells, ranging in number from 500 to 6,000,000, counted in a blood-counting chamber. These were injected subcutaneously in an attempt to ascertain the approximate number of cells and the time required to produce a tumor of the size obtained with the fractionated material.

3. Eighty smears were made of the entire chromatin fraction of 45 gm. of tumor tissue. These were stained with Wright's stain and examined.

RESULTS

AAF-induced Hepatoma

The pertinent data are presented in Table 1.

[2] We are greatly indebted to Dr. James B. Murphy, of the Rockefeller Institute, for these tumors.

No intact cells, nuclei, or recognizable cell fragments were observed in any of the stained smears or paraffin sections of the mitochondria fraction. Invariably, structures resembling mitochondria appeared to be the only particulate bodies encountered in this fraction. Their structure was particularly well preserved when the sucrose technic was employed (Fig. 1).

No cells or nuclei were seen in the entire mitochondria fraction of 100 gm. of normal liver, suspended in saline containing a little methylene blue and examined in a blood-counting chamber.

Hepatomas were found at the site of injection (liver) in 2 of 118 animals injected with the mitochondria fraction. One of these (Figs. 2–4) had been prepared by the saline (4) and the other (Figs. 5–7) by the sucrose (6) technic. The former was found 30 days and the latter 60 days after injection.

TABLE 1

Tumors following Intrahepatic Injection of AAF-induced Hepatoma Cells and Cell Fractions

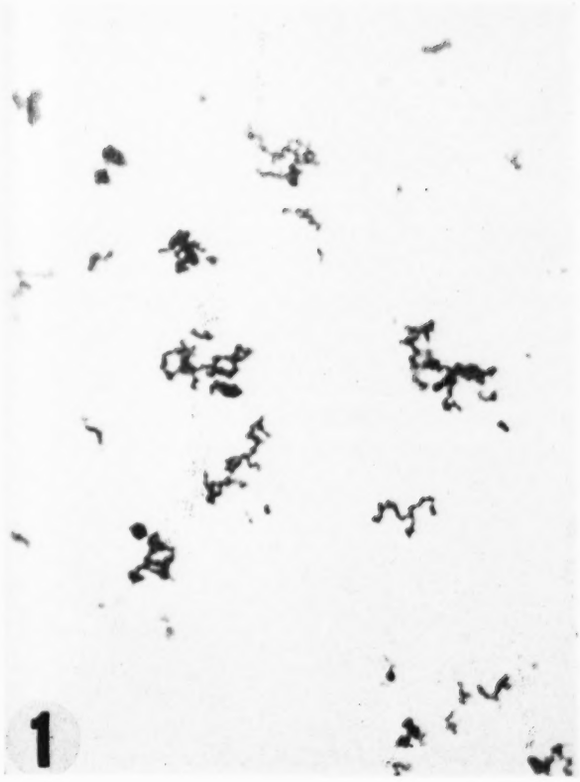
Material	Total injections	Tumors
Intact Cells	23	0
Mitochondria (saline)	45	1
Mitochondria (sucrose)	73	1
Chromatin	15	0

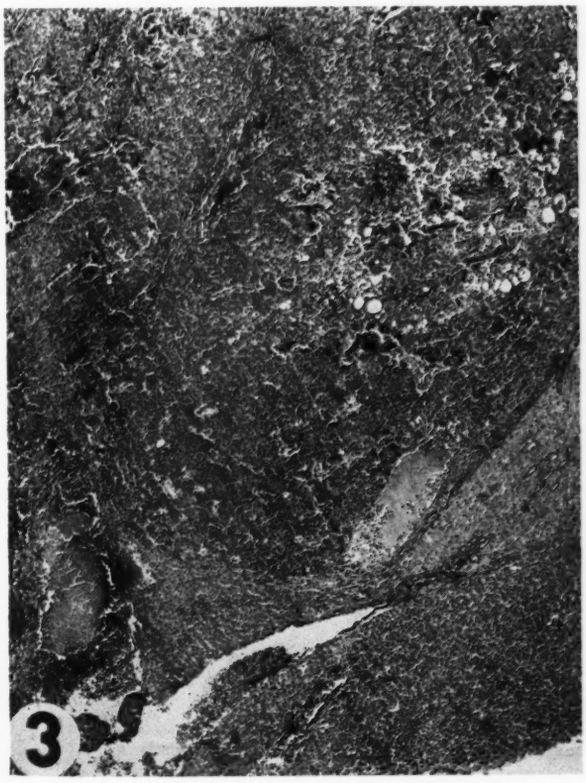
Morphology of lesions.—

1. Rat 1914 (Wistar male, stock diet): The liver of this animal was inoculated with the mitochondria fraction of a hepatoma obtained from a Wistar rat after 232 days on a diet containing 0.03 per cent 2-acetaminofluorene. The recipient animal was killed 30 days later. At the site of injection there was a pale, gray-brown nodule, measuring 1.2 × 1.0 × 1.0 cm. (Fig. 2). Grossly, the liver was otherwise normal.

On microscopic examination, the nodule presented the appearance of a typical AAF-induced hepatoma (3) (Figs. 3 and 4). There was complete obliteration of the normal liver architecture. The lesion was composed of atypical epithelial cells, varying in size, shape, and nuclear chromatin content. The majority were oval or polyhedral, with basophilic cytoplasm and large nuclei, many of which were hyperchromatic. There were numerous typical and atypical mitotic forms. The cells were generally irregularly arranged, but occasionally showed a cordlike arrangement.

There were hemorrhagic and necrotic areas in the marginal portion of the nodule. Sections from other areas of the liver showed a moderate degree of parenchymatous and fatty change, with well preserved lobular architecture.





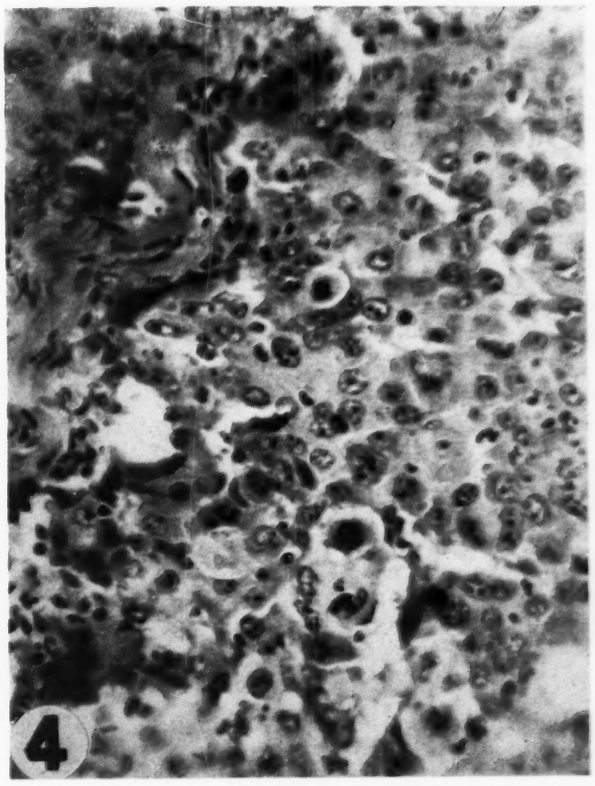
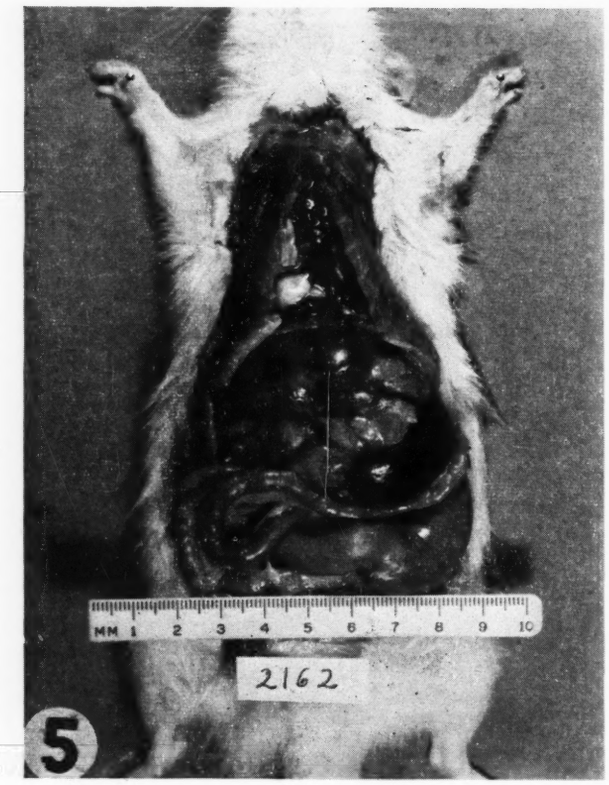


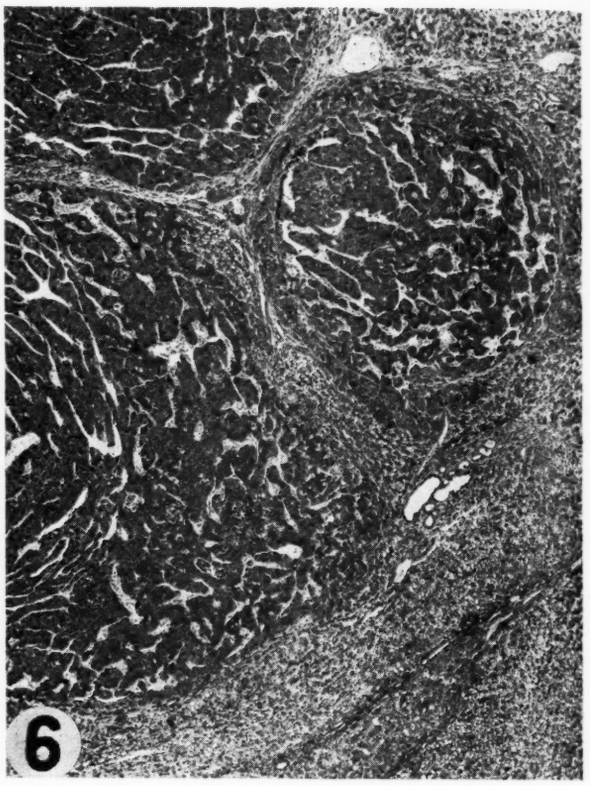
FIG. 1.—AAF-hepatoma mitochondria. ×1,000

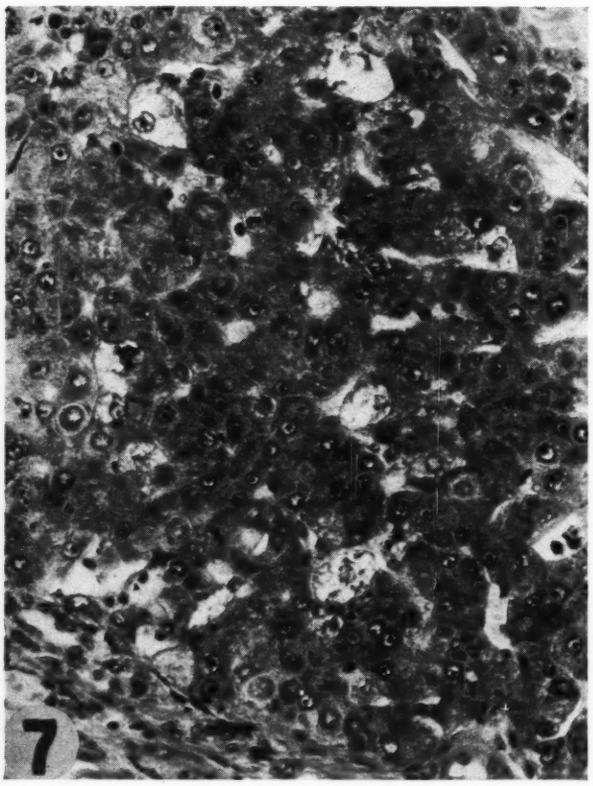
FIG. 2.—Hepatoma induced by intrahepatic injection of AAF-hepatoma mitochondria 30 days previously.

Fig. 3.—Marginal portion of tumor in Figure 2. ×35

FIG. 4.—Higher magnification of section of tumor in Figure 2. ×460.







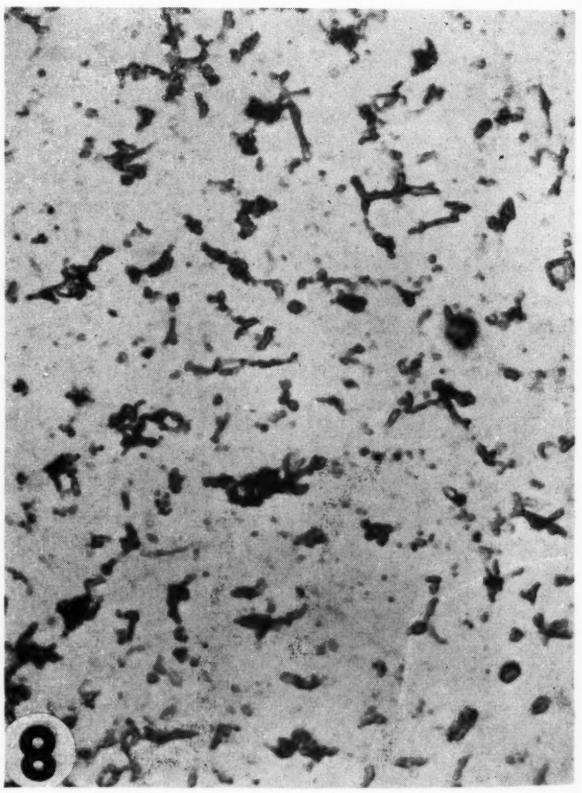


FIG. 5.—Hepatoma induced by intrahepatic injection of AAF-hepatoma mitochondria 60 days previously.

FIG. 6.—Marginal portion of tumor in Figure 2

FIG. 7.—Higher magnification of section of tumor in Figure 2. ×460.

FIG. 8.—Lymphosarcoma chromatin. ×600

2. Rat 2161 (Wistar male, stock diet): The liver of this animal was injected with the mitochondria fraction of several pooled hepatomas obtained from three rats after 240–280 days on a diet containing 0.03 per cent 2-acetaminofluorene. The recipient animal was killed 60 days later. At the site of injection there were several confluent yellow-gray nodules, measuring about 1.5 × 1.0 × 0.8 cm. (Fig. 5). On section, the cut surface was light gray with occasional areas of hemorrhage. The remainder of the liver appeared normal grossly.

On microscopic examination (Figs. 6 and 7), the nodules presented the characteristic appearance of certain AAF-induced hepatomas, with areas of necrosis and inflammatory reaction. One portion consisted of atypical epithelial cells arranged in irregular cords. The cells varied in size and shape, being polygonal, oval, or elongated. The cytoplasm was basophilic, and the nuclei showed variations in size, shape, and chromatin content. There were many large, hyperchromatic nuclei; multinucleated giant cells and abnormally large cells with deeply stained, lobulated nuclei were also seen. Few mitotic forms were noted. Large portions of the nodules were necrotic, the adjacent areas showing an inflammatory reaction with cirrhosis.

No tumors were observed after intrahepatic injection of suspensions of intact hepatoma cells, cell-residue fractions, chromatin fractions, or suspensions of 2-acetaminofluorene crystals in rat plasma.

Murphy Rat Lymphosarcoma

The pertinent data regarding the incidence of tumors and leukemia in animals which received injections of various preparations in different tissues are presented in Table 2. Tumors developed in two of three animals at the site of intrahepatic injection of a suspension of intact cells (not included in the table).

In the animals which received subcutaneous injections, tumors were usually palpable (in positive cases) in 3–4 days when intact cells were employed, in 3–7 days with the chromatin fraction, and in 7–18 days with the mitochondria fraction. The animals were killed after 10–30 days, the size of the tumors in those receiving intact cells and those receiving cell fractions being essentially the same.

It was found that a suspension of at least 3,000 intact cells in 1-ml. NaCl solution, injected subcutaneously, was required to produce a tumor that approximated the rate of growth of the slowest growing tumor after injection of chromatin fractions.

In the case of several of the tumors indicated in Table 2 as developing after subcutaneous injection of cell suspensions and of chromatin fractions, the injected material was derived from tumors that had developed after subcutaneous injection of chromatin fractions.

No intact cells or nuclei or recognizable cytoplasmic or nuclear fragments (other than chromatin threads) were seen in any of the smears of the injected chromatin fractions. The only recognizable particulate bodies were the chromatin threads (Fig. 8). The same statements apply to the 80 smears that represented the entire chromatin fraction of 45 gm. of tumor tissue.

TABLE 2

TUMORS FOLLOWING INJECTION OF LYMPHOSARCOMA CELLS AND CELL FRACTIONS

Material	Site of injection								
	Subcutaneous			Bone-marrow		Spleen		Thymus	
	Total	Tumor	Leukemia	Total	Tumor	Total	Tumor	Total	Tumor
Intact Cells	153	114	13	2	2	4	1	1	1
Chromatin	80	19	13	5	2	2	0	3	0
Mitochondria	58	3	1	8	0	5	0	4	0
Chromatin and mitochondria	9	4	2						

Morphology of lesions.—These tumors presented a remarkably uniform appearance; there was no significant difference between those induced by injection of intact cell suspensions (Fig. 9) and those which followed injection of cell fractions (Figs. 10–12).

The tumors consisted chiefly of closely packed cells resembling medium-sized lymphocytes. The cells were round, oval, or polyhedral, with a rather scant, basophilic cytoplasm, often forming a narrow rim around the nucleus. The nuclei were large, hyperchromatic, and sometimes indented. The nuclear membranes were distinct, and there were many mitotic figures. Abnormally large cells were seen occasionally.

There were occasional areas of hemorrhage. The stroma was scant and consisted of a faintly outlined fibrillary network containing delicate blood vessels. There was frequent evidence of invasion of adjacent tissues (fat, muscle, bone).

DISCUSSION

The significance of these observations rests upon the establishment of two fundamental

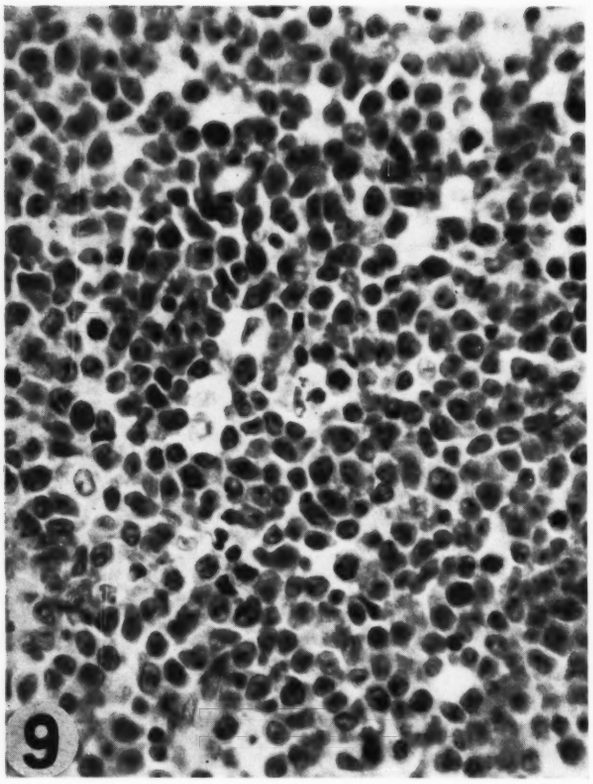


FIG. 9.—Murphy rat lymphosarcoma induced by subcutaneous injection of suspension of intact tumor cells 11 days previously. ×400.

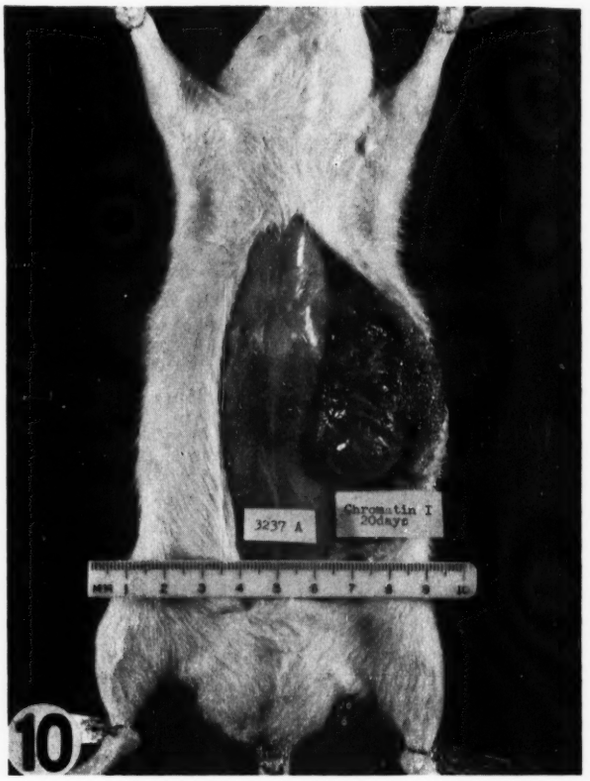


FIG. 10.—Murphy rat lymphosarcoma induced by subcutaneous injection of chromatin of lymphosarcoma cells 20 days previously.

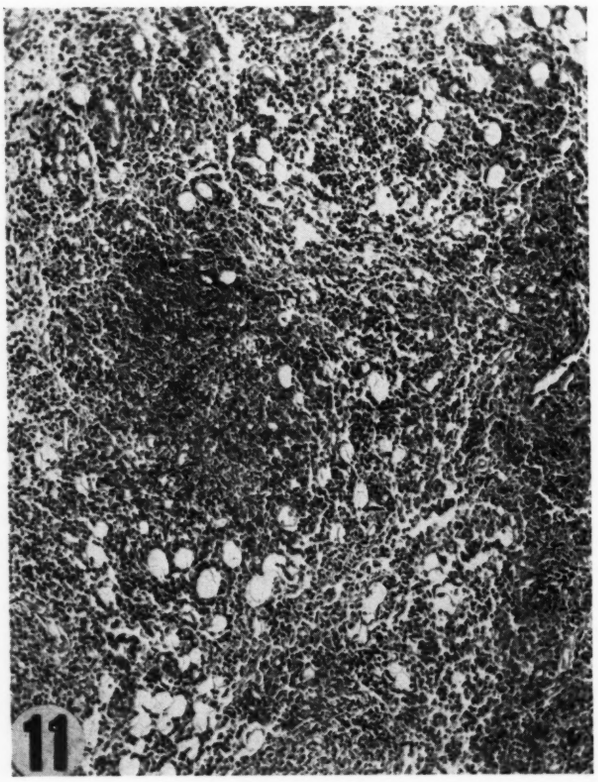


FIG. 11.—Section of tumor in Figure 10. ×65

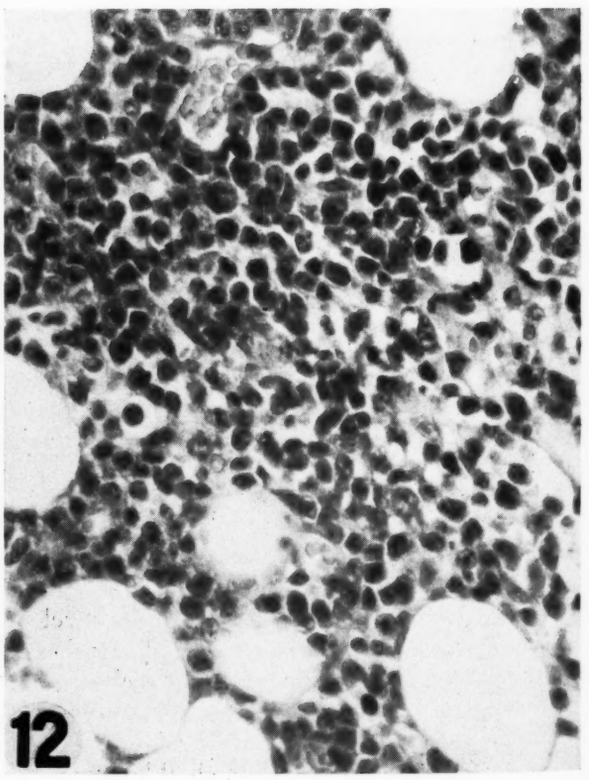


FIG. 12.—Higher magnification of section of tumor in Figure 10. ×400.

points: (*a*) the neoplastic nature of the induced lesions and (*b*) the absence of intact tumor cells in the injected mitochondria and chromatin fractions.

In the case of the AAF-induced hepatoma, proof of the neoplastic nature of the lesion is difficult. In an extensive experience with this tumor in the rat, we have not been able to transplant it successfully. Bielschowsky (1) reported its successful transplantation into the Wistar rat in three instances. Establishment of the neoplastic nature of the lesions which developed in two animals at the site of intrahepatic injection of mitochondria fractions must depend upon their cytological characteristics, which were identical with those of hepatomas produced by oral administration of 2-acetaminofluorene (3).

There can be no doubt of the neoplastic nature of the lesions that followed subcutaneous injection of chromatin and mitochondria fractions of the Murphy rat lymphosarcoma. Their gross appearance, rate of growth, and cytological features seem to be characteristic. Definite proof is afforded by the fact that identical lesions were produced by subcutaneous injection of both cell suspensions and chromatin fractions of the chromatin-induced tumors. These "third-generation" lesions were indistinguishable from the original tumors.

We know of no way in which contamination of the mitochondria and chromatin fractions with intact cells can be excluded with absolute certainty. Direct proof of the absence of cells in these fractions is impossible. We believe, however, that the following observations indicate the improbability of such contamination:

1. Not a single recognizable cell, nucleus, or cell fragment was seen in smears or paraffin sections of the 118 mitochondria and 15 chromatin fractions of hepatoma cells and the 58 mitochondria and 80 chromatin fractions of lymphosarcoma cells injected into the recipient animals in this study.

2. No cells or nuclei were seen in the entire mitochondria fraction of 100 gm. of normal liver, prepared in the same manner as the hepatoma mitochondria fraction.

3. No cells or nuclei were seen in the entire chromatin fraction of 45 gm. of lymphosarcoma tissue.

4. When intact lymphosarcoma cells were added to the suspended chromatin fraction and the mixture was recentrifuged at 1,500 *g* for 10 minutes, intact cells could be readily identified in smears. It seems unlikely that such cells would have consistently escaped detection in the examination of smears and paraffin sections made routinely from every chromatin fraction.

We feel that it is highly improbable that the chromatin and mitochondria fractions were contaminated with intact cells. The evidence appears to warrant the tentative conclusion that the tumors that appeared after injection of the chromatin fractions of lymphosarcoma cells were induced by this material, presumably by entrance of the chromatin strands or some component of these structures into lymphocytes of the recipient animals.

The fact that only 3 tumors developed in 58 animals which received injections of the mitochondria fraction of lymphosarcoma cells, in contrast to the high incidence in those receiving the chromatin fraction, raises a question as to the possibility of contamination of the mitochondria with chromatin fragments. Such contamination is indeed probable, inasmuch as the mitochondria fractions were prepared after preliminary homogenation of the tissue in a Waring Blendor, a procedure which causes fragmentation of nuclei. Chromatin strands and fragments would then be incorporated into the mitochondria fraction sedimented at 24,000 *g*, inasmuch as they probably would not have been removed by the preceding centrifugation at 600 *g*. The positive results obtained with mitochondria fractions may therefore have been due to chromatin material. It is interesting in this connection, however, that tumors developed in 44 per cent of nine animals injected subcutaneously with a mixture of mitochondria and chromatin, as compared with 24 per cent of those receiving chromatin alone and 5 per cent of those receiving mitochondria alone. The striking difference in incidence of tumors induced by fractions of hepatoma and lymphosarcoma cells may be explained on the basis of the difference in transplantability of these tumors in the rat.

There was a rather striking difference in the incidence of leukemia (infiltration of liver and kidney) in animals that developed local tumors following subcutaneous injection of intact cells (11 per cent leukemia) and in those receiving cell fractions (chromatin, 70 per cent; mitochondria, 33 per cent; chromatin and mitochondria, 50 per cent). A difference of this magnitude and in this direction would not be anticipated if the development of the malignant lesions in the recipient animals were due to contamination of the cell fractions with intact cells.

If these observations are valid, they indicate that malignant potentialities of neoplastic cells reside in the chromatin material and may be transmitted to normal cells, probably by entrance of

the chromatin threads or some component of these structures into the cells. The significance of mitochondria in this connection is open to question.

Experiments are in progress in which attempts are being made to insure destruction of cells by various means without altering the structure or activity of chromatin and mitochondria.

SUMMARY

Lymphosarcomas developed in rats at the site of subcutaneous and intramedullary injection of chromatin and mitochondria fractions of the Murphy rat lymphosarcoma. Leukemia occurred in a high percentage of animals which developed local tumors following subcutaneous injection of lymphosarcoma cell fractions.

Hepatomas developed in two rats at the site of intrahepatic injection of the mitochondria fraction of AAF-induced rat hepatomas. This fraction may have been contaminated with chromatin fragments.

Direct proof of the absence of intact cells in these fractions is impossible, but data presented seem to indicate that such contamination is highly improbable.

REFERENCES

1. Bielschowsky, F. Distant Tumors Produced by 2-Amino and 2-Acetyl-aminofluorene. Brit. J. Exper. Path., **25**:1–4, 1944.
2. Bryan, W. R.; Kahler, H.; and Riley, V. T. Attempts To Demonstrate a Virus-like Principle in Mammalian Tumors by the Yolk-Sac Injection Technique. AAAS Research Conference on Cancer, pp. 40–53, 1945.
3. Cantarow, A.; Paschkis, K. E.; Stasney, J.; and Rothenberg, M. S. The Influence of Sex Hormones upon the Hepatic Lesions Produced by 2-Acetaminofluorene. Cancer Research, **6**:610–16, 1946.
4. Claude, A. Fractionation of Mammalian Liver Cells by Differential Centrifugation. J. Exper. Med., **84**:51–89, 1946.
5. Claude, A., and Potter, J. S. Isolation of Chromatin Threads from the Resting Nucleus of Leukemic Cells. J. Exper. Med., **77**:345–53, 1943.
6. Hogeboom, G. H.; Schneider, W. C.; and Pallade, G. E. The Isolation of Morphologically Intact Mitochondria from Rat Liver. Proc. Soc. Exper. Biol. & Med., **65**:320–21, 1947.
7. Taylor, A.; Hungate, R. E.; and Taylor, R. Yolk-Sac Cultivation of Tumors. Cancer Research, **3**:537–41, 1943.
8. Taylor, A., and Kynette, A. The Virus Production of Mammalian Tumors. AAAS Research Conference on Cancer, pp. 24–37, 1945.
9. Twombly, G. H. Virus Induction of Mammalian Tumors. AAAS Research Conference on Cancer, pp. 56–57, 1945.
10. Murphy, J. B., and Sturm, E. The Transmission of an Induced Lymphatic Leukemia and Lymphosarcoma in the Rat. Cancer Research, **1**:379–83, 1941.

The Role of Cellular Fractions in the Transplantation of the Walker Rat Carcinoma 256*

W. W. Tourtellotte, Ph.D., and J. B. Storer, M.D.

(From the University of Chicago Toxicity Laboratory, University of Chicago, Chicago, Ill.)

Stasney, Paschkis, and Cantarow (6) have reported that hepatomas may develop in rats that have received intrahepatic injections of a cell-free mitochondria fraction obtained from 2-acetaminofluorene-induced rat hepatomas. They found two hepatomas at 29 and 35 days, respectively, in 36 rats injected with this mitochondria fraction. Intrahepatic injection of intact tumor cells or crystals of 2-acetaminofluorene suspended in serum produced no tumors in 48 rats. The present investigation was designed to determine whether cellular fractions from a tumor not chemically induced would incite malignancy when injected as a cell-free inoculum.

MATERIALS AND METHODS

The transplantable Walker rat carcinoma 256 was used in this study. This tumor originally arose as a spontaneous breast carcinoma and has been consecutively transplanted in rats for many years. Histologically, it has the appearance of an undifferentiated carcinoma with some sarcomatous characteristics. Ordinarily, 95–100 per cent of rats receiving subcutaneous injections of cell suspensions develop the tumor. It grows rapidly, mestastasizes to regional lymph nodes relatively late, and kills usually within 30 days after injection.

The method of Hogeboom *et al.* (3), with sucrose as a dispersing medium, was employed to obtain various cellular fractions. Under sterile conditions, 5.5 and 10.5 gm. of tumor in the first and second experiments, respectively, were homogenized in 9 volumes of cold 0.25 M sucrose immediately after aseptic dissection from three donor rats. (In the first experiment 20,000 units of crystalline penicillin G [Lilly] were added to the homogenate.) All but 5.0 ml. were centrifuged in the cold room (3° C.) at 1,500 *g* for 10 minutes to precipitate the nuclei, together with small numbers of intact cells which were not disrupted during homogenization. The nuclei were washed twice for 10 minutes with 3.0 ml. of sucrose solution. The washings and supernatant solution of the nuclei were combined and centrifuged at 10,800 *g* for 15 minutes and washed twice with 3.0 ml. of sucrose solution for 10 minutes to sediment the mitochondria. Centrifugation at 21,000 *g* for 1 hour of the resulting supernatant solution, together with the washings of mitochondria, yielded a precipitate of submicroscopic material (the "Microsomes" of Claude [2]). The microsomes were washed once with 3.0 ml. of sucrose solution and centrifuged at 21,000 *g* for 1 hour. The final supernatant solution and washing were saved for injection.

The efficacy of the separation procedure has been shown for liver tissue (2, 3, 5); however, it is important to demonstrate the reliability of the method for this tumor. Smears of the various resuspended fractions were stained with Wright's stain and Janus green B. The nuclei fraction contained intact cells. The exact proportion was difficult to estimate accurately because of the presence of many nuclei with variable amounts of adherent cytoplasm. The mitochondria fraction contained a number of nuclei, but no intact cells were seen. The microsome fraction was found to be free from mitochondria.

The supernatant solution of the microsomes doubtless contained particulate material which could be sedimented at higher centrifugal speeds; Chantrenne (1) stated that the microsomes range in size from very small units of nearly pure ribonucleo-protein to relatively large aggregates of nucleo-proteins and other proteins.

A total of 90 Sprague-Dawley rats (45 males and 45 females), weighing 250–350 gm., were divided into groups of 10, each group consisting of 5 males and 5 females. The hair over the back of each rat was clipped, the skin was painted with Merthiolate [R], and under ether anesthesia the various fractions were injected subcutaneously.

* The work described in this paper was conducted at the University of Chicago Toxicity Laboratory under a research contract supported by the Atomic Energy Commission and administered through the Medical Division, Chemical Corps, U.S. Army. Under the terms of the contract neither the Atomic Energy Commission nor the Army Chemical Corps is responsible for the opinions or conclusions of the authors.

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The volumes of each fraction given per injection per group of ten rats in the first experiment were: 0.25 ml. of 10 per cent homogenate, 0.25 ml. of mitochondria suspension (mitochondria residue suspended in 6.0 ml. of 0.25 M sucrose solution), 0.5 ml. of microsome suspension (residue suspended in 6.0 ml. of sucrose solution), and 2.0 ml. of the final supernatant (70 ml.). In the second experiment, the volumes of each fraction injected were: 0.25 ml. of homogenate, 0.5 ml. of nuclei suspension (nuclei residue suspended in 6.0 ml. of sucrose solution), 0.5 ml. of mitochondria suspension, 0.5 ml. of microsome suspension and 10.0 ml. of final supernatant (140 ml.). The rats were checked weekly for tumors. Observations were continued for 6 weeks.

RESULTS

Tumors developed in six of the ten injection sites in the first experiment and in nine of the ten injection sites in the second experiment, in the groups receiving the tumor homogenate. Tumors also developed in eight of the ten injection sites in the second experiment in the group receiving the nuclei suspension which was known to contain intact cells.

The second experiment was designed to increase the concentration of the suspension and the amount of suspension injected. Since all the residues were resuspended in 6 ml. of isotonic sucrose solution, there was a twofold increase in residue concentration. The volume per injection was increased twofold for the mitochondria suspension and fivefold for the final supernatant solution. In spite of the increase in concentration of the suspensions and the increase of volume per injection, no tumors developed in the groups receiving mitochondria suspension containing nuclei, microsome suspension, or final supernatant solution.

DISCUSSION

The only successful transplantations occurred with the crude homogenate and the nuclei fraction, both of which contained intact cells. Although no attempt was made to prepare nuclei free of intact cells, evidence in these studies indicates that nuclei are not sufficient for the successful transplantation of the Walker rat carcinoma 256. Since the mitochondria fraction which contained both nuclei and mitochondria failed to produce "takes," neither of these is sufficient for successful transplantation. If this fraction had produced tumors it would be impossible, of course, to tell which was responsible, and further refinement of the technic of separation would have been necessary.

While it is theoretically possible to transplant a tumor by injection of one intact cell into a susceptible animal, it has been our experience with this particular tumor that a large number of cells must be injected to insure success of the transplant. Hence, no significance is attached to the difference in incidence of tumors in rats injected with the homogenate in the first experiment (60 per cent) as compared to the higher incidence (90 per cent) in the second experiment.

We have found that bacterial contamination of the inoculum may interfere with the transplantation of this tumor. To control this factor, penicillin was added to the homogenate in the first experiment, and sterile precautions were employed in both experiments. Since no infections developed in any of the 90 injection sites, there was no evidence of bacterial contamination of the cellular fractions.

With the possible exception of certain tumors of virus etiology (4), successful transplantation of neoplasms is probably due to proliferation of the cells inoculated into the recipient. The cells of the recipient animal do not take part in the production of the tumor mass except to provide vascularity and a supporting stroma. The view that this is the mechanism involved in the successful transplantation of the Walker rat carcinoma 256 is supported by our data.

Cell-free mitochondria fractions from chemically induced hepatomas appear, from the results of Stasney *et al.* (6), to have the ability to induce malignancy. In view of the fact that transplantability to other animals of the same strain is a fairly generally accepted criterion of malignancy of tumors of mice and rats, it is surprising that Stasney and co-workers did not obtain tumors in the rats injected with intact tumor cells.

SUMMARY

Five- and 10-gm. portions of a Walker rat carcinoma 256 were fractionated by ultra-centrifugation into subcellular components immediately after dissection from three donor rats. Only the fractions containing intact cells produced tumors when injected subcutaneously into rats. The mitochondria fraction which was known to contain nuclei, the microsome fraction, and the supernatant of the microsomes produced no tumors over a 6-week observation period.

REFERENCES

1. CHANTRENNE, H. Recherches sur des particulates cytoplasmiques de dimensions macromoléculaires piches en acide pentosenucléique. II. Relations avec les fermentes respiratoires. Enzymologia, **11**:213–21, 1943–45.
2. CLAUDE, A. Fractionation of Mammalian Liver Cells by Differential Centrifugation. I. Problems, Methods, and Preparation of Extract. J. Exper. Med., **84**:51–89, 1946.

3. HOGEBOOM, G. H.; SCHNEIDER, W. C.; and PALLADE, G. E. Cytochemical Studies of Mammalian Tissues. I. Isolation of Intact Mitochondria from Rat Liver; Some Biochemical Properties of Mitochondria and Submicroscopic Particulate Material. J. Biol. Chem., **172**:619–35, 1948.
4. ROUS, P. The Virus Tumors and the Tumor Problem. Harvey Lectures, **31**:74–115, 1935–36.
5. SCHNEIDER, W. C. Intracellular Distribution of Enzymes. III. The Oxidation of Octanic Acid by Rat Liver Fractions. J. Biol. Chem., **176**:259–66, 1948.
6. STASNEY, J.; PASCHKIS, K. E.; and CANTAROW, C. Hepatomas Following Intrahepatic Injection of "Mitochondria Fraction" of Chemically Induced Hepatomas. Cancer Research, **9**:617–18, 1949.

The Influence of Riboflavin, Pyridoxine, Inositol, and Protein Depletion-Repletion upon the Induction of Neoplasms by Choline Deficiency*

A. E. Schaefer, D. H. Copeland, W. D. Salmon, and O. M. Hale

(From the Department of Animal Husbandry and Nutrition, Agricultural Experiment Station of the Alabama Polytechnic Institute, Auburn, Ala.)

The production of neoplasms in the livers and in other tissues of rats as a result of prolonged choline deficiency was reported by Copeland and Salmon in 1946 (1). This was confirmed by Engel, Copeland, and Salmon in 1947 (4) and by Staub, Viollier, and Werthemann in 1948 (10). The influence of the vitamin-B complex on the incidence of liver tumors in rats fed carcinogenic dyes has been studied by many laboratories. The observation by Kensler *et al.* (6) in 1941 that riboflavin suppressed the development of tumors in rats fed *p*-dimethylaminoazobenzene, has been amply confirmed. Retardation of the production of azo dye-induced tumors by diets low in pyridoxine was suggested by Miner *et al.* (8) in 1943 and by Miller *et al.* (7) in 1945. It was shown by Engel (2) that inositol, along with choline, was a necessary dietary constituent for the maintenance of liver fat at normal levels. Recently, it was observed in this laboratory (5) that protein depletion-repletion superimposed upon choline deficiency in adult rats greatly increased liver fat in very short periods.

It is the purpose of this paper to describe the influence of variations in the dietary level of riboflavin, pyridoxine, and inositol, and of protein depletion-repletion upon the incidence of choline deficiency-induced tumors in rats.

EXPERIMENTAL

In the experiments designed to determine the influence of riboflavin, pyridoxine, and inositol on the neoplasm induction by chronic choline deficiency, weanling rats of the Alabama Experiment Station (AES) strain, weighing 40–50 gm., were used. For the protein depletion-repletion studies, female rats weighing 160 gm. and male rats weighing 200 gm. were used. All animals were housed in individual cages and uniformly grouped with respect to number, weight, sex, and litter.

Feed and water were supplied *ad libitum*. The basal diet contained extracted peanut meal, 30 (3); sucrose, 39.5; extracted casein, 6 (9); minerals, 4.4 (undried) (9); L-cystine, 0.1; cod liver oil, 1; and lard, 19. Vitamins were added in mg/kg of diet, except where otherwise indicated, as follows: thiamine, 2; riboflavin, 4; pyridoxine, 2; calcium pantothenate, 10; niacin, 20; inositol, 200; alpha-tocopherol, 25; alpha-tocopherol acetate, 25; and 2-methyl-1,4-naphthoquinone, 5. The dietary variations of riboflavin, pyridoxine, inositol, and choline, and the numbers of animals per treatment, are given in Tables 1, 2, and 3.

The diet fed during protein depletion was similar to the basal diet described, except that the peanut meal and casein were replaced by sucrose. During the repletion period, the animals were fed the basal diet. The variations in the periods of protein depletion and repletion and the choline chloride supplementation are given in Table 4.

Since the hepato-renal damage is severe and mortality is high in choline-deficient weanling rats, their diets were supplemented with 800 mg. choline chloride/kg for the first 2 weeks, and this was then reduced by 200 mg. at 2-week intervals. Under such a procedure only 2 of 78 choline-deficient rats died of the acute deficiency. Of the fourteen groups fed various low choline diets, four groups received a continued dietary supplement of 200 mg of choline chloride/kg, to determine whether this sub-minimal level would prolong survival and yield a higher tumor incidence.

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TABLE 1

Effect of Riboflavin on Liver Cirrhosis and Tumor Incidence in Choline Deficiency

Diet variation		Av. time on expt. (weeks)	Av. final body weight (gm.)	Hb. at 40 weeks (gm. per cent)	Gross liver cirrhosis*	Tumor incidence*	No. and types of tumors
Riboflavin (mg/kg)	Choline (mg/kg)						
1	0	61	214	13.8	7/8	4/8	3 hepatomas 1 sarcoma
2	0	80	237	12.1	4/4	2/4	2 hepatomas
2	200	61	219	14.0	2/3	0/3	
20	0	61	240	14.9	6/8	0/8	
1	2,000	83	314	15.4	0/4	0/4	
20	2,000	70	348	15.4	0/4	0/4	

* Ratio of affected animals/total animals in group.

TABLE 2

Effect of Pyridoxine on Liver Cirrhosis and Tumor Incidence in Choline Deficiency

Diet variation		Av. time on expt. (weeks)	Av. final body weight (gm.)	Hb. at 40 weeks (gm. per cent)	Gross liver cirrhosis	Tumor incidence	No. and types of tumors
Pyridoxine (mg/kg)	Choline (mg/kg)						
0	0	60	112	8.8	7/8	3/8	3 hepatomas
0.5	0	82	162	13.1	4/4	2/4	2 hepatomas
0.5	200	63	243	13.4	1/3	1/3	1 mammary
1	0	70	220	12.5	8/8	3/8	3 hepatomas
10	0	82	256	12.9	4/6	2/6	1 hepatoma 1 sarcoma
20	0	66	257	12.1	7/8	5/8	3 hepatomas 1 mammary 1 thoracic
0.5	2,000	94	300	15.6	0/4	0/4	
10	2,000	82	368	15.7	0/4	0/4	

TABLE 3

Effect of Inositol on Liver Cirrhosis and Tumor Incidence in Choline Deficiency

Diet variation		Av. time on expt. (weeks)	Av. final body weight (gm.)	Hb. at 40 weeks (gm. per cent)	Gross liver cirrhosis	Tumor incidence	No. and types of tumors
Inositol (mg/kg)	Choline (mg/kg)						
0	0	77	234	12.0	4/4	2/4	2 hepatomas
0	200	73	295	14.3	2/4	1/4	1 hepatoma*
200	0	69	305	11.5	4/4	2/4	2 hepatomas
200	200	59	241	13.6	3/4	2/4	2 hepatomas
0	2,000	79	380	15.6	0/4	0/4	
200	2,000	82	361	17.7	0/4	0/4	

* One rat in this group developed diverticulosis of the stomach, not classified as a tumor.

TABLE 4

Effect of Protein Depletion-Repletion on Liver Cirrhosis and Tumor Incidence in Choline Deficiency in Young Adult Rats

Choline supplement (mg/kg)	Feeding cycle: Depletion (weeks)	Feeding cycle: Repletion (weeks)	No. rats	Av. time on expt. (weeks)	Av. final body weight (gm.)	Liver cirrhosis	Tumor incidence	No. and types of tumors
0	2	3	8*	47	132	8/8	3/8	3 hepatomas
0	3	3	2†	49	155	2/2	1/2	1 hepatoma
2,000	2	3	9	71	361	0	0/9	

* One of the eight rats was maintained on the protein depletion-repletion regimen for 3 cycles and thereafter continued on the repletion diet. This rat developed a hepatoma.

† These rats were maintained on the repletion diet after 3 cycles of protein depletion.

Tissues were saved for routine microscopic studies. Bouin's fixative and hematoxylin and eosin stain were routinely used.

Hemoglobin was determined by the acid hematin method twice during the experiment, at 32 and 40 weeks.

RESULTS

Growth, survival, and hemoglobin levels.—The average final body weight of 76 weanling rats fed diets low in choline, with varying levels of riboflavin, pyridoxine, and inositol, was 226 gm. as compared to 345 gm. for the control rats receiving 0.2 per cent choline chloride. In general, the animals receiving the choline-deficient diets grew moderately well the first 6 months and thereafter declined in weight very slowly with a marked loss just prior to death.

The average survival time of the choline-deficient rats was 68 weeks, with a range of 42–98 weeks. A number of these animals had lung involvement, characterized by râles and hemorrhagic rhinitis. The controls were maintained for an average of 82 weeks. The hemoglobin level of the 62 rats receiving no choline supplement averaged 12.4 gm. per cent. Of these animals, one group of eight rats receiving 20 mg of riboflavin/kg of diet had an average hemoglobin level of 14.9 gm. per cent, which was essentially normal for the control animals fed 0.2 per cent choline chloride. Supplementation of various diets with the low level of 0.02 per cent choline chloride resulted in an average hemoglobin level of 13.8 gm. per cent (Tables 1–3).

The ten adult rats subjected to protein depletion-repletion without choline supplementation survived an average of 47 weeks, with a range of 25–63 weeks. Final body weight of these animals was 136 gm. and ranged from 81 to 194 gm. The nine control rats, similarly treated but receiving 0.2 per cent dietary choline chloride, were maintained on the experiment for an average of 71 weeks and weighed 361 gm. (Table 4).

Incidence of tumors and liver cirrhosis.—In the riboflavin, pyridoxine, and inositol experiments involving a total of 76 choline-deficient rats, 29 had tumors, and 63 had gross cirrhosis of the liver. No tumors or cirrhotic livers were found in 24 control rats receiving 0.2 per cent dietary choline chloride (Tables 1–3). In the protein depletion-repletion studies, 4 of the 10 choline-deficient rats had tumors, and all had cirrhotic livers. The 9 control animals did not develop liver cirrhosis or tumors (Table 4).

Influence of riboflavin.—A strikingly significant result of this series of experiments was the suppression of tumor development by 20 mg of riboflavin/kg of diet. Of fifteen animals that received 1 or 2 mg of riboflavin/kg of diet (Table 1), 40 per cent had tumors, whereas none of the eight rats fed high riboflavin developed tumors. Although gross liver cirrhosis was observed in six of the rats fed high levels of riboflavin, it was much less severe than that noted in the other choline-deficient animals. The hemoglobin level of this group was normal.

Influence of pyridoxine.—The omission of pyridoxine from the basal diet markedly suppressed growth. The average maximum weight attained was approximately 120 gm., and the final body weight averaged 112 gm. (range, 96–151 gm.). Typical pyridoxine-deficiency symptoms were noted. Although growth was greatly suppressed, three of the eight rats developed tumors (Table 2). This group also had the lowest hemoglobin level (8.8 gm. per cent). Of 23 rats receiving 0, 0.5, and 1 mg of pyridoxine/kg of diet, 39 per cent had tumors (Table 2), as compared to 50 per cent incidence in 14 rats receiving 10 and 20 mg. of pyridoxine. Thus, in general, the level of pyridoxine did not influence tumor incidence.

Influence of inositol.—The omission or inclusion of dietary inositol did not affect tumor incidence (Table 3). The diet containing 200 mg of inositol/kg is comparable to diet 43A used in the studies reported by Copeland and Salmon (1). The latter workers found a tumor incidence of 58 per cent, as compared to 44 per cent in this experiment.

Influence of protein depletion-repletion.—Protein depletion-repletion markedly shortened the time required for the development of neoplasms in young rats, as compared to the time required for weanling rats. The adult rats of this series of experiments survived an average of 47 weeks and tumors appeared in 32 weeks, whereas the weanling rats of the previous series of experiments survived an average of 68 weeks and tumors appeared in 42 weeks. All the choline-deficient animals had liver cirrhosis, and 40 per cent had neoplasms (Table 4). Body weight of the deficient animals declined rapidly during protein depletion with partial recovery during repletion. In general, body weight was progressively lowered from an average of 180 gm. at the start of the experiment to 136 gm., final weight. The control rats that received 0.2 per cent dietary choline chloride and were subjected to protein depletion-repletion lost weight during depletion, but more than regained this weight loss during repletion and attained a final body weight of 361 gm.

The results indicated that the protein depletion-repletion treatment need not be so drastic as that

employed for most of the animals in these experiments. One of the eight rats undergoing protein depletion for 2-week periods was maintained on the repletion diet after a total of three depletions. This animal survived for 63 weeks and had a hepatoma. The two rats subjected to protein depletion for 3 weeks were only depleted 3 times and were thereafter maintained on the repletion diet. One of the two rats developed a hepatoma.

Histopathology.—Neoplasms were found in 33 of 86 of all the chronic choline-deficient rats in 32–98 weeks. Of these neoplasms, 28 were classified as hepatoma, 2 as mammary carcinoma, and 2 as sarcoma. One thoracic tumor has not yet been identified.

The gross appearance of a typical liver tumor is illustrated in Figure 1. This tumor measured 2.5 cm. in diameter. The liver tumors encountered in this experiment varied from 1 to 4 cm. in diameter and were mutiple in some livers.

Microscopic studies revealed that, in addition to tumors, about half of the livers exhibited lesions similar to the one illustrated in Figure 2. This type of lesion was classified as a multiple cyst. These cysts contained small amounts of fibro-purulent exudate and were lined by flattened endothelium-like cells in some cases and cuboidal and columnar cells in others. Considerable interstitial connective tissue and a focal loss of parenchymal cells were characteristic of these areas.

Bile duct proliferation was a common finding and was especially extensive in the livers of the 28 rats that developed hepatomas. A type of cholangioma is shown in Figure 3. This cholangioma developed in the liver of a rat, in addition to a hepatoma. The typical acini formation and adenomatous nature of this lesion, as well as the characteristic connective tissue stroma, is evident.

The hepatomas presented very interesting cell patterns, which are illustrated by a series of photomicrographs. Figure 4 shows a hepatoma characterized by chains of cells and irregular trabeculae. "Gland-like" formations such as these were found in most of the hepatomas. The arrangement of the connective tissue stroma in a liver tumor that has the appearance of an adenocarcinoma is shown in Figure 5. The cells are arranged in primitive fashion, simulating glandular stratified epithelial elements. Numerous mitotic figures were observed, and the anaplastic nature of the tumor is evident.

The gross appearance of a mammary carcinoma is shown in Figure 6. A photomicrograph of a section of this tumor is illustrated in Figure 7. The tumor tissue appears to be invading the skeletal muscle. This tumor was composed of small epithelial cells with round nuclei arranged in gland-like structures. In some areas the cells were closely packed, and the glandular arrangement was lost. These tumors were extremely cellular, and mitotic figures were common.

The cellular nature of a sarcoma is shown in Figure 8. The tumor is composed of closely packed spindle cells. The nuclei are stained dark, have a characteristic oval shape, and contain one or more distinct nucleoli. The cytoplasm is fusiform to stellate and without distinct borders. The cells are arranged in different planes and whorls and often have a combed appearance.

DISCUSSION

The role of choline deficiency in carcinogenesis has been further substantiated. Neoplasms occurred in 33 of 86 rats that were fed choline-deficient diets for 32–98 weeks, while no tumors or liver cirrhosis were found in 33 control rats that received 0.2 per cent dietary choline chloride.

An interesting observation was the indication that high riboflavin suppressed tumor formation in chronic choline deficiency. The absence of tumors in eight rats (from six litters) fed a choline-deficient diet supplemented with 20 mg of riboflavin/kg indicates that this vitamin exerts a protection similar to that shown in the case of the azo dye-induced tumors (6). This is puzzling in view of the fact that choline does not prevent the development of azo dye-induced tumors. High levels of riboflavin in the diet did not afford as complete protection to the liver as choline, since 75 per cent of these rats developed a mild liver cirrhosis.

Varying the level of pyridoxine or inositol did not influence tumor incidence Of interest was the observation that neoplasms developed in rats fed a diet without pyridoxine and choline supplementation, even though body weight was only 33–50 per cent of normal.

The production of neoplasms in nearly mature rats subjected to protein depletion-repletion in addition to choline deficiency and the complete protection by choline against such a rigorous treatment further emphasize the important role of choline. One would suspect that a procedure which results in caloric restriction such as protein depletion would suppress tumor development; however, with protein depletion-repletion, tumors actually appear in only two-thirds of the time required to produce tumors in weanling rats fed a choline-deficient diet without the depletion-repletion procedure.

The lowering of hemoglobin level in the choline-deficient rats confirms the observation by Engel (3). No correlation could be made between hemo-

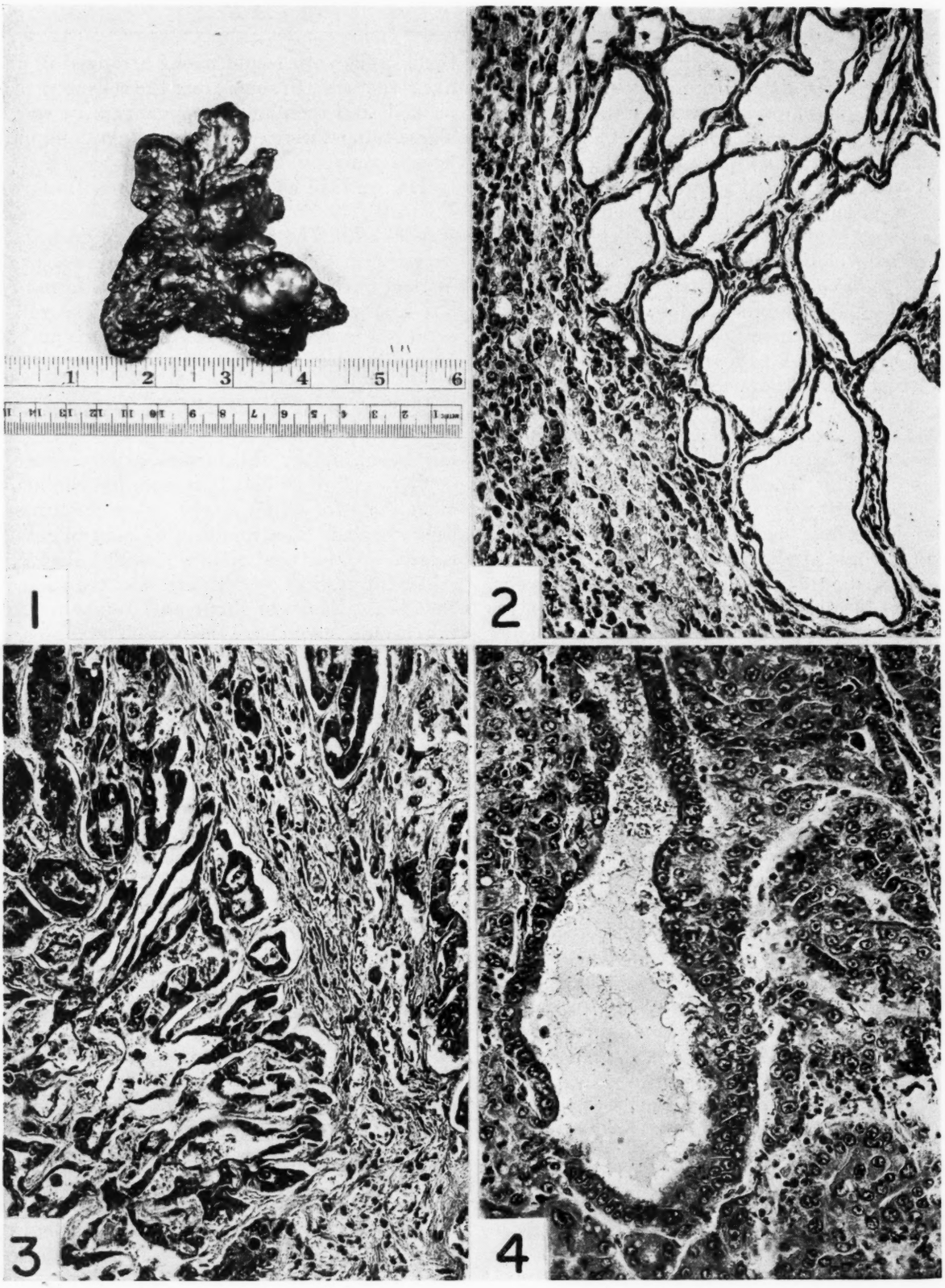

FIG. 1.—Photograph showing the typical gross appearance of a hepatoma. This tumor developed in the liver of a rat fed a choline-deficient diet containing 20 mg of pyridoxine/kg of diet for 82 weeks. Reduced to less than one-half actual size.

FIG. 2.—Photomicrograph showing multiple cysts in the liver of a rat fed a choline-deficient diet containing 10 mg of pyridoxine/kg of diet. The cystic spaces are lined with flattened endothelium-like cells. Hematoxylin and eosin stain. ×230.

FIG. 3.—Photomicrograph of a cholangioma of the liver of a rat fed a choline-deficient diet containing no inositol for 85 weeks. Hematoxylin and eosin stain. × 230.

FIG. 4.—Photomicrograph of a hepatoma of the liver of a rat fed a choline-deficient diet containing no inositol for 72 weeks. Notice the "glandlike" formation and typical cellular nature of this hepatoma. Hematoxylin and eosin stain. × 230.

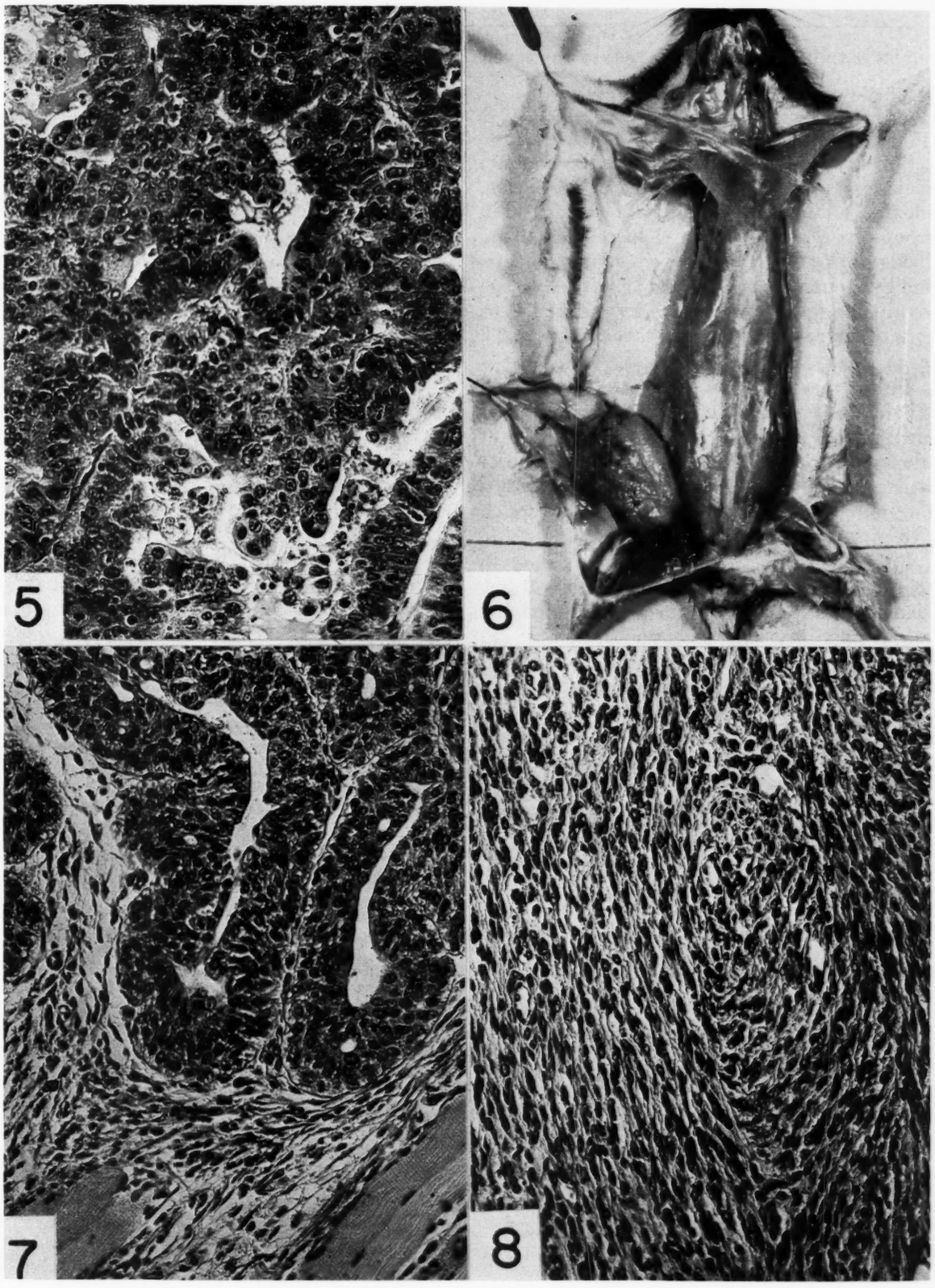

FIG. 5.—Photomicrograph of a liver tumor that has the appearance of an adenocarcinoma. This tumor developed in a rat fed a choline-deficient diet without inositol for 72 weeks. Hematoxylin and eosin stain. × 230.

FIG. 6.—Photograph illustrating the gross appearance of a mammary tumor in a rat fed a choline-deficient diet supplemented with 0.5 mg of pyridoxine/kg of diet for 48 weeks. Reduced to about one-half the actual size.

FIG. 7.—Photomicrograph of a section of the mammary tumor shown in Figure 6. The tumor appears to be invading the skeletal muscle. Hematoxylin and eosin stain. × 230.

FIG. 8.—Photomicrograph of a section of a sarcoma. This tumor developed in a rat fed a choline-deficient diet containing 1 mg of riboflavin/kg of diet for 44 weeks. Hematoxylin and eosin stain. × 230.

globin level and tumor incidence within any given treatment.

SUMMARY

1. Seventy-six weanling rats were fed choline-deficient diets with varying levels of riboflavin, pyridoxine, and inositol. Neoplasms were found in all groups except one group of eight rats fed 20 mg of riboflavin/kg of diet. Of the remaining 68 rats, 57 had liver cirrhosis and 29 had tumors. Average survival time was 68 weeks. No liver cirrhosis or tumors occurred in 24 control rats receiving 0.2 per cent dietary choline chloride.

2. Under these experimental conditions, high riboflavin (20 mg/kg of diet) prevented choline deficiency-induced neoplasms. Varying levels of pyridoxine or inositol had no influence on tumor incidence. Neoplasms developed in rats receiving a choline- and pyridoxine-deficient diet, even though growth was suppressed.

3. Four of ten young adult rats subjected to protein depletion-repletion and chronic choline deficiency developed neoplasms; all had cirrhotic livers. Average survival time was 47 weeks. No tumors or cirrhotic livers were found in nine controls fed 0.2 per cent dietary choline chloride.

4. Hepatomas were found in 28 of the 33 rats with neoplasms. Of the remaining neoplasms, there were two mammary carcinomas, two sarcomas, and one thoracic tumor.

REFERENCES

1. Copeland, D. H., and Salmon, W. D. The Occurrence of Neoplasms in the Liver, Lungs, and Other Tissues of Rats as a Result of Prolonged Choline Deficiency. Am. J. Path., **22**:1059–79, 1946.
2. Engel, R. W., The Relation of B-Vitamins and Dietary Fat to the Lipotropic Action of Choline. J. Nutrition, **24**: 175–85, 1942.
3. ———. Anemia and Edema of Chronic Choline Deficiency in the Rat. J. Nutrition, **36**:739–50, 1948.
4. Engel, R. W.; Copeland, D. H.; and Salmon, W. D. Carcinogenic Effects Associated with Diets Deficient in Choline and Related Nutrients. Ann. New York Acad. Sc., **49**:49–67, 1947.
5. Hale, O. M. The Effect of Protein Depletion-Repletion upon Chronic Choline Deficiency in Rats. M.S. Thesis, Ala. Polytech. Inst., 1950.
6. Kensler, C. J.; Sugiura, K.; Young, N. F.; Halter, C. R.; and Rhoads, C. P. Partial Protection of Rats by Riboflavin with Casein against Liver Cancer Caused by Dimethylaminoazobenzene. Science, **93**:308–10, 1941.
7. Miller, E. C.; Baumann, C. A.; and Rusch, H. P. Certain Effects of Dietary Pyridoxine and Casein on the Carcinogenicity of *p*-Dimethylaminoazobenzene. Cancer Research, **5**:713–16, 1945.
8. Miner, D. L.; Miller, J. A.; Baumann, C. A.; and Rusch, H. P. The Effect of Pyridoxine and Other B-Vitamins on the Production of Liver Cancer with *p*-Dimethylaminoazobenzene. Cancer Research, **3**:296–302, 1943.
9. Salmon, W. D. Some Physiological Relationships of Protein, Fat, Choline, Methionine, Cystine, Nicotinic Acid, and Tryptophane. J. Nutrition, **33**:155–68, 1947.
10. Staub, H. von; Viollier, G.; and Werthemann, A. Über des auftreten von multiplen Adenomen in der Leber von cholinarm ernährten Ratten. Experientia, **6**:233–36, 1948.

The Synthesis of Citrulline and *p*-Aminohippuric Acid by Rat Hepatoma*

TA CHENG TUNG, M.D., PH.D.,† AND PHILIP P. COHEN, PH.D., M.D.

(From the Laboratory of Physiological Chemistry, University of Wisconsin, Madison 6, Wis.)

The capacity of cell-free preparations of neoplastic tissue to carry out synthetic reactions has not been widely investigated. The desirability of such studies is apparent, since information might be provided which would bear on the integrated metabolic functions of the cell, particularly in relation to the coupling of oxidative energy with the synthetic reactions. Previous studies in this laboratory dealing with the synthesis of citrulline (2, 3) and *p*-aminohippuric acid (4–6), suggested conditions suitable for the study of these synthetic reactions in neoplastic liver preparations. While the syntheses of these compounds represent highly specialized functions of the normal liver cell, their dependence on energy coupling reactions make their study in neoplastic liver tissue particularly desirable.

The reactions investigated in this study were the following:

1. Glutamic acid + CO_2 + NH_3 + ornithine→ citrulline.
2. Carbamylglutamic acid + NH_3 + ornithine→citrulline.
3. *p*-Aminobenzoic acid + glycine→*p*-aminohippuric acid.

EXPERIMENTAL

Animals and liver tumors.—Liver tumors were induced[1] by feeding *p*-dimethylaminoazobenzene (0.06 per cent of diet) to Sprague Dawley strain rats (Group III) using diet No. 3 of Miller *et al.* (11). Two groups of control animals were used; one was fed the same diet as the experimental but without added *p*-dimethylaminoazobenzene (Group II), and another group consisted of animals fed a stock commercial diet (Purina Dog Chow) (Group I). The animals weighed between 185 and 350 gm. and were fed *ad libitum*. The animals were examined for the presence of tumors after 4 months of feeding the experimental diet. When tumors were found, the animals were fed the control diet (II) until sacrificed.

Preparation of enzyme systems.—Washed residue preparations of normal liver were prepared as previously described (6). Livers from *p*-dimethylaminoazobenzene-fed animals were separated by dissection into grossly distinguishable neoplastic and non-neoplastic tissue and were used for preparation of washed residue in the same manner as employed for normal tissue. Neoplastic tissue was dissected free of necrotic tissue before use. Two gm. of tissue were homogenized with 5 ml. of isotonic KCl solution and filtered through a double layer of gauze. The homogenate was then centrifuged for 10 minutes at 2,000 *g* and the supernate discarded. The residue was then twice washed with 5-ml. portions of isotonic KCl solution. The washed residue was finally suspended in 10 ml. of isotonic KCl solution. An aliquot of 1 ml., containing between 1 and 1.5 mg. of nitrogen, was added to each flask. All manipulations were carried out in the cold room, and the tissues were kept in an ice bath up to the time of incubation.

Measurement of citrulline synthesis.—The general procedures employed were those used by Cohen and Grisolia (2). Two sets of experiments were conducted in each case, one in which glutamic acid was present and the other in which carbamylglutamic acid replaced glutamic acid. This was done to determine the capacity of neoplastic tissue to synthesize the postulated intermediate from glutamic acid, CO_2, and ammonia. In all instances the systems were incubated aerobically at 38° C. in Warburg flasks for 20 minutes in the absence of ornithine, which was then added from the side arm and the complete system then allowed to incubate for 10 minutes. The reaction was stopped by the addition of 4 ml. of 5 per cent perchloric acid and citrulline determined on aliquots of the filtrate according to the method of Archibald (1). The results are expressed as micromoles of citrul-

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† Fellow of the Chinese Ministry of Education.

[1] We are indebted to Dr. Sam Sorof of this laboratory for making available to us the animals fed the experimental diets.

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line formed per milligram of washed residue nitrogen.

Measurement of p-*aminohippuric acid synthesis.*—The general procedure was that employed by Cohen and McGilvery (6). The incubations were carried out aerobically at 38° C. in Warburg flasks for 45 minutes. The reaction was stopped by the addition of 4 ml. of 0.2 N trichloroacetic acid per 1 milliliter of incubation mixture, and an aliquot of the filtrate was taken for analysis of *p*-aminohippuric acid.

Substrates and reagents.—The preparations and sources of substrates and reagents have been previously described (2, 6).

RESULTS

The amount of citrulline and *p*-aminohippuric acid synthesis, with the use of different preparations, is shown in Table 1. In confirmation of previous findings by Cohen and Grisolia (2), it is seen that carbamylglutamic acid yields more citrulline

TABLE 1

SYNTHESIS OF CITRULLINE AND *p*-AMINOHIPPURIC ACID IN NORMAL AND NEOPLASTIC RAT LIVER

No. of rat	Group	Citrulline: Glutamic acid system μM/mg N	Citrulline: Carbamylglutamic acid system μM/mg N	Ratio	*p*-Aminohippuric acid μM/mg N
1		1.02	1.28		1.12
2		0.63	1.51		0.97
3		0.49	1.20		1.20
4		1.13	1.85		1.16
5		1.00	2.79		0.84
6	I	0.72	1.86		0.81
7		0.59	1.37		1.03
8		0.77	1.88		0.88
9		0.61	1.41		0.92
10		0.72	2.25		1.22
11		0.71	1.37		1.15
12		0.73	1.03		1.05
		Ave. (0.76)	Ave. (1.65)	2.2	Ave. (1.03)
13		0.30	0.94		0.88
14		0.24	0.78		1.29
15		0.29	1.63		0.98
16		0.25	1.43		1.15
17	II	0.22	0.96		0.83
18		0.23	0.98		0.82
19		0.26	1.08		1.01
20*		0.19	0.82		1.03
21*		0.16	0.91		0.87
		Ave. (0.23)	Ave. (1.06)	4.1	Ave. (0.98)
22		0.39	0.70		0.45
23		0.15	0.47		0.72
24		0.07	0.92		0.65
25		0.13	0.76		0.58
26	III, A	0.25	1.06		0.54
27		0.17	0.35		0.67
28	(non-neo-	0.21	1.10		0.64
29	plastic	0.52	1.12		0.72
30	tissue)	0.14	0.92		0.22
31		0.15	0.93		0.35
		Ave. (0.21)	Ave. (0.83)	3.9	Ave. (0.55)
22		0.15	0.50		0.36
23		0.13	0.45		0.24
24		0.06	0.84		0.14
25	III, B	0.09	0.65		0.06
26		0.26	0.78		0.11
27	(neoplas-	0.09	0.27		0.08
28	tic tis-	0.07	0.31		0.09
29	sue)	0.11	0.41		0.11
30		0.09	0.27		0.01
31		0.07	0.34		0.04
		Ave. (0.11)	Ave. (0.48)	4.4	Ave. (0.12)

* Given parenteral injection of 200 μg. of riboflavin per day for 10 days.

Citrulline synthesis: Final substrate concentration as follows: Magnesium sulfate, 6×10^{-3} M; potassium phosphate at pH 7.15, 1.3×10^{-2} M; fumarate, 3×10^{-2} M; L-glutamic acid, or carbamyl-L-glutamic acid, 1×10^{-2} M; DL-ornithine, 2.5×10^{-3} M; ATP 1.2×10^{-3} M; ammonium chloride 4×10^{-3} M; sodium bicarbonate, 5×10^{-3} M; and KCl to bring the medium to isotonicity. The total volume was 4.0 ml.

p-Aminohippuric acid synthesis: Final substrate concentration as follows: *p*-Aminobenzoic acid, 1×10^{-3} M; glycnei, 1.5×10^{-2} M; magnesium sulfate, 2.6×10^{-3} M; cytochrome c, 1.2×10^{-6} M; fumarate, 2.5×10^{-3} M; ATP, 5×10^{-4} M; and KCl, 4×10^{-2} M. The total volume of 4.0 ml. was made isotonic with potassium phosphate at pH 7.55.

than does glutamic acid under the same conditions with normal liver. This is also true of the experiments in which liver preparations from animals on the special diets, with and without *p*-dimethylaminoazobenzene, were used. It is to be noted, however, that the ratio of citrulline synthesis from carbamylglutamic acid, as compared with synthesis from glutamic acid, is different for the different groups. This is shown in Column 5, Table 1. Thus, while it is clear from Table 1 that the synthesis of citrulline is lowest in the case of neoplastic tissue, whether glutamic acid or carbamylglutamic acid is used, citrulline synthesis was also low in the case of livers from Group II and the grossly non-neoplastic tissue from Group III, A. These data indicate that the loss of enzyme activity in neoplastic tissue is greater for the step leading to the synthesis of the intermediate carbamyl derivative than for the step leading to the synthesis of citrulline from this intermediate. Of interest, however, is the observation that the ratio of citrulline synthesis from carbamylglutamic acid and glutamic acid is of the same order for the systems prepared from livers of animals on the diets of Groups II and III. It would appear that the diet in Groups II and III is deficient in some factor which is functioning as coenzyme for the enzymatic step concerned with the conversion of glutamic acid to the carbamyl derivative. Since the diet employed is low in riboflavin, two animals, Nos. 20 and 21, were injected with 200 μg. of riboflavin daily for 10 days. As can be seen from Table 1, this was without effect. MacLeod *et al.* (10) have reported that livers from animals on a biotin-deficient diet have a lowered capacity to synthesize citrulline in a system containing glutamic acid. However, the rate of synthesis in biotin-deficient animals is the same as in the normal, using carbamylglutamic acid (8). However, the diet employed in the present study was apparently adequate in biotin, and, further, the animals showed no signs suggesting such a deficiency. It would thus appear that although some factor, as yet unknown and which is apparently absent in the diets of Groups II and III, is responsible for some of the loss of enzymatic activity, the neoplastic process results in an additional decrease in enzyme activity, of the order of 50 per cent (compare Group III, A and B).

The findings in the case of *p*-aminohippuric acid synthesis (Table 1) clearly indicate that there is no difference in the amount of synthesis by liver from animals on the two diets, Groups I and II. On the other hand, the feeding of *p*-dimethylaminoazobenzene results in a decrease in activity of approximately 50 per cent (Group III, A), while the activity of neoplastic tissue is decreased to very low values, of the order of 12 per cent of normal.

DISCUSSION

Since the washed residue preparations used in the present study consist chiefly of mitochondria and nuclei and since the synthetic activity is confined chiefly to the former (unpublished studies from this laboratory), it is of interest to estimate the relative enzymatic activity of normal and hepatoma tissue. Price *et al.* (12) have found that per gram of fresh tissue, normal liver yields about 2.5 times as much mitochondria protein as does hepatoma. Assuming that this factor obtains in the fresh tissue, the low values for enzymatic synthesis observed with washed residue prepared from hepatoma, as compared with normal liver, would be still lower (by factor of 2.5) when compared on a fresh weight basis.

While previous experience with the enzyme preparations from normal liver indicates that the incubation conditions employed in this study were such as to permit quantitative assay, the possibility exists that the washed residue preparations from hepatoma have a limiting factor other than the enzyme concentration which is not provided for in the medium employed. In view of this, the quantitative differences observed in synthetic activity of normal liver, as compared with hepatoma, should not be accepted with finality. A more adequate evaluation of quantitative differences would require assays at different time intervals and at different enzyme concentrations.

Previous studies by Greenstein (9) with slices of rat hepatoma revealed that the synthesis of urea from citrulline was impaired. It is clear from the present study that the steps leading to citrulline synthesis from ornithine are also impaired, with the primary enzymatic steps leading to the formation of the carbamyl intermediate quantitatively more affected than the subsequent reactions.

While the synthesis of *p*-aminohippuric acid is known to be impaired in cases of liver damage (7), it is clear from the present study that the decrease in synthetic activity in the neoplastic tissue is greater than that observed in the adjacent grossly non-neoplastic tissue.

Since it is generally characteristic of tissues undergoing neoplastic transformation to lose their more specialized functions, it is perhaps not surprising that hepatoma tissue should show a loss in enzymatic activity concerned with the synthesis of citrulline and *p*-aminohippuric acid. On the other hand, the synthesis of *p*-aminohippuric acid represents a model system for peptide bond synthesis (4), and, if the *in vivo* synthesis of protein involves the step-wise synthesis of peptide chains, it might

be expected that the synthesis of *p*-aminohippuric acid would be increased if the same enzyme systems were involved. In this connection, Zamecnik *et al.* (14) have reported that slices of hepatoma show a higher rate of protein synthesis, as measured by incorporation of isotopically labeled amino acids, than slices of normal tissue. On the other hand, Rittenberg and Shemin (13), using whole animals, have reported contrary findings.

The mechanism by which oxidative energy is coupled with synthetic reactions to meet the thermodynamic requirements of such endergonic systems is not known. The fortification of the systems investigated in this study with adenosine triphosphate has provided a source of high energy which is required for the reaction to proceed. In addition, these systems contained fumarate which can be utilized by liver preparations from normal animals for the regeneration of the high energy bond nucleotides (2, 6). These studies have not established whether the failure in neoplastic tissue is that of the coupling mechanisms between the high energy-bond compounds formed by oxidation and the enzymatic steps concerned specifically with the synthesis. Since both synthetic systems, when fortified with adenosine triphosphate,[2,3] have been shown to be catalyzed by soluble enzymes free of oxidative enzymes, this possibility can be investigated by the use of such preparations from neoplastic tissue.

SUMMARY

1. Washed residue preparations of *p*-dimethylaminoazobenzene-induced hepatomas show a decrease in citrulline synthesis to approximately 14 and 30 per cent of normal in the presence of glutamic acid and carbamylglutamic acid, respectively. There is thus a relatively greater loss of enzyme activity in hepatoma tissue concerned with the conversion of glutamic acid to the carbamyl intermediate than for the conversion of the latter of citrulline. This loss of activity is due in part to the deficiency of some unknown essential in the diet of the experimental animals.

2. The synthesis of *p*-aminohippuric acid from *p*-aminobenzoic acid and glycine by washed residue prepared from hepatoma decreased to a level approximately 12 per cent of that of a similar preparation from normal liver.

3. The significance of these findings is discussed.

[2] S. Grisolia and P. P. Cohen, unpublished studies.

[3] R. W. McGilvery and P. P. Cohen, unpublished studies.

REFERENCES

1. Archibald, R. M. Determination of Citrulline and Allantoin and Demonstration of Citrulline in Blood Plasma. J. Biol. Chem., **156**:121–42, 1944.
2. Cohen, P. P., and Grisolia, S. The Role of Carbamyl-L-Glutamic Acid in the Enzymatic Synthesis of Citrulline from Ornithine. J. Biol. Chem., **182**:747–61, 1950.
3. Cohen, P. P., and Hayano, M. The Enzymatic Steps in Urea Synthesis. J. Biol. Chem., **172**:405–15, 1948.
4. Cohen, P.P., and McGilvery, R. W. I. The Formation of *p*-Aminohippuric Acid by Rat Liver Slices. J. Biol. Chem., **166**:261–72, 1946.
5. ———. II. The Formation of *p*-Aminohippuric Acid by Liver Homogenates. *Ibid.*, **169**:119–36, 1947.
6. ———. III. On the Mechanism of *p*-Aminohippuric Acid Synthesis. *Ibid.*, **171**:121–33, 1947.
7. Deiss, W. P., and Cohen, P. P. Studies in *p*-Aminohippuric Acid Synthesis in the Human: Its Application as a Liver Function Test. J. Clin. Investigation, **29**: 1014–20, 1950.
8. Feldott, G.; MacLeod, P.; and Lardy, H. A. Site of the Influence of Biotin on Citrulline Synthesis. Fed. Proc., **9**: 170, 1950.
9. Greenstein, J. P. Incubation of Citrulline and Ammonia with Normal and Neoplastic Hepatic Tissues. J. Nat. Cancer Inst., **3**:293–96, 1942.
10. MacLeod, P.; Grisolia, S.; Cohen, P. P.; and Lardy, H. A. III. The Synthesis of Citrulline from Ornithine in Liver Tissue and Biotin-deficient Rats. J. Biol. Chem., **180**:1003–11, 1949.
11. Miller, E. C.; Miller, J. A.; Kline, B. E.; and Rusch, H. P. Correlation of the Level of Hepatic Riboflavin with the Appearance of Liver Tumors in Rats Fed Aminoazo Dyes. J. Exper. Med., **88**:89–98, 1948.
12. Price, J. M.; Miller, J. A.; Miller, E. C.; and Weber, G. M. Studies on the Intracellular Composition of Liver and Liver Tumor from Rats Fed 4-Dimethylaminoazobenzene. Cancer Research, **9**:96–102, 1949.
13. Rittenberg, D., and Shemin, D. In Green, D. E. Currents in Biochemical Research, p. 272. New York: Interscience Publishers, Inc. 1946.
14. Zamecnik, P. C.; Frantz, I. D., Jr.; Loftfield, R. B.; and Stephenson, M. L. Incorporation *in Vitro* of Radioactive Carbon from Carboxyl-labelled DL Alanine and Glycine into Proteins of Normal and Malignant Rat Liver. J. Biol. Chem., **175**:299–314, 1948.

Mammary Excretion of 20-Methylcholanthrene*

HARRY SHAY, M.D., BERNICE FRIEDMANN, M.A., MARGOT GRUENSTEIN, AND SIDNEY WEINHOUSE, Ph.D.

(From the Lankenau Hospital Research Institute and the Institute for Cancer Research, and the Fels Research Institute of Temple University, Philadelphia, Pa.)

Recently, evidence was reported for the belief that a carcinogenic agent, 20-methylcholanthrene, could be secreted into the milk of a lactating animal and thereby gain entrance into the bodies of the offspring to cause an abnormally high incidence of neoplasms (1). Further positive evidence for this mode of excretion of methylcholanthrene has now been obtained by means of ultraviolet absorption spectroscopy, a method which has been used widely to follow the distribution of this and other carcinogens in animal tissues (2).

In the detection of methylcholanthrene in tissues of rats, advantage was taken of its high absorption peaks at 284 and 295 mμ. A solution of 1 μg. per milliliter in petroleum ether has an optical density (log 100/per cent transmission) of .250 at 284 and .312 at 295 mμ (measurements made with a Beckmann model DU spectrometer). The sensitivity of this method is limited by the fact that the unsaponifiable matter of rat tissues displays rather high general absorption in the ultraviolet, which increases with decreasing wave length (Fig. 1). As shown in Figure 1, however, the addition of methylcholanthrene in a concentration of 0.1 μg. per milliliter can be readily detected by inflections in the absorption curve of such an extract. Since the absorption of solutions of the unsaponifiable matter of a whole rat is such that it can be conveniently measured in a dilution of about 100 ml., the presence of methylcholanthrene in the bodies of rats in the amount of 10 μg. should be detectable by inflections at 284 and 295 mμ; and amounts of approximately 50 μg. should appear as discernible peaks on the absorption curve. In the present work, inflections at 284 and 295 mμ were taken as qualitative evidence for the presence of methylcholanthrene, with the recognition, of course, of the possibility that part or all of the absorption at these wave lengths may be due to metabolic products of methylcholanthrene.

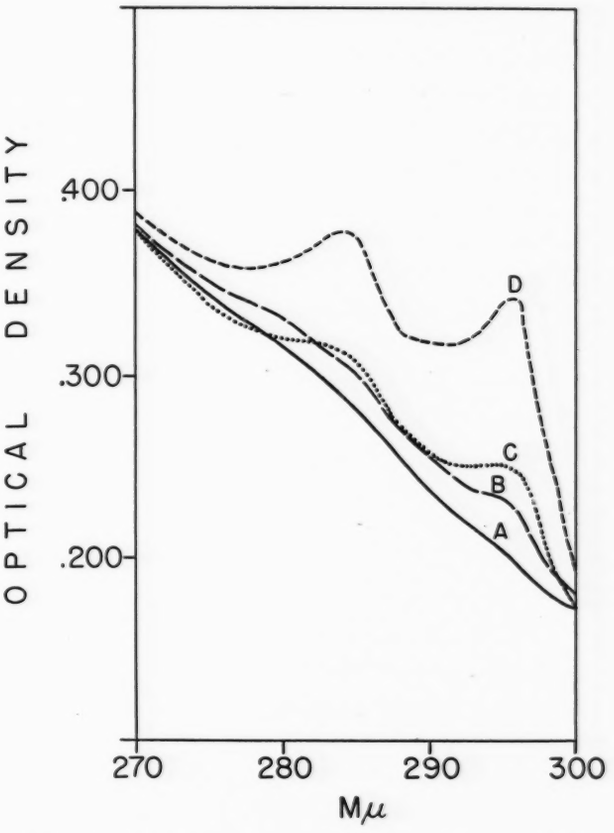

FIG. 1.—Absorption curves produced by adding to a petroleum ether solution of the unsaponifiable matter of a rat carcass (curve *A*) 0.1, 0.2, and 0.5 μg. of 20-methylcholanthrene per milliliter of solution (curves *B*, *C*, and *D*, respectively).

METHODS AND RESULTS

Oral administration.—A female rat 24 hours *post partum* received 8 mg. of methylcholanthrene by stomach catheter in 1 ml. of olive oil, and this dose was repeated daily for 10 days. During this period a good deal of care was taken to prevent contamination of the suckling rats with the maternal feces. The possibility of the ingestion of maternal feces by rats 10 days of age seemed rather re-

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mote, since at this age they show no interest in food other than maternal milk. To minimize such a possibility, however, the animals were kept on wide-mesh screen, and any feces which remained were removed on daily inspection of the cages. To avoid including any of the carcinogen which may have contaminated the skin of the young rats through possible contact with maternal feces, special care was taken, during skinning, to avoid contact of the skinned carcass with the fur. Twenty-four hours after the last administration, when the

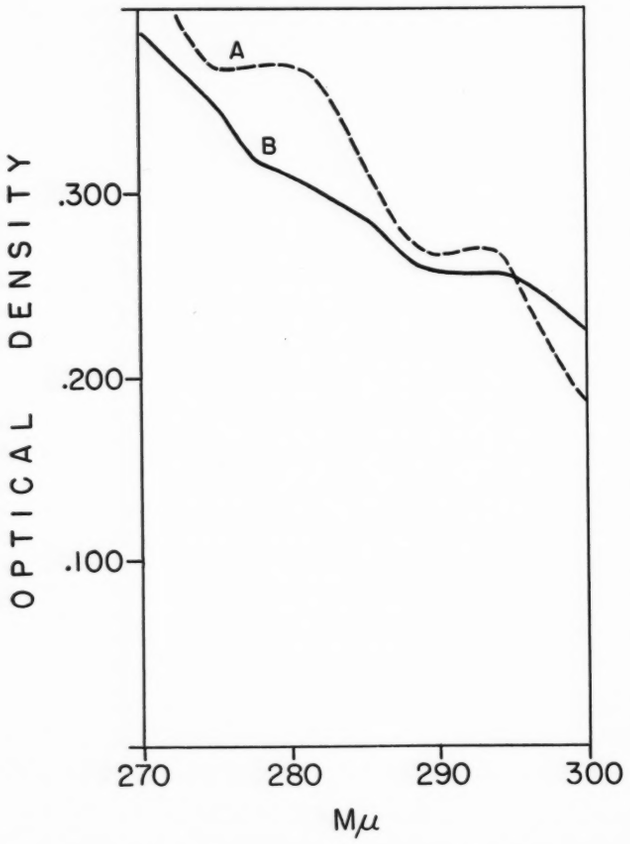

FIG. 2.—Absorption curve of extract of unsaponifiable matter of bodies of young whose mother received methylcholanthrene by oral instillation. Curve *A*, extract of young; curve *B*, extract of mother.

young were 12 days old, the mother and her litter of ten were sacrificed. The mother was carefully skinned and the entire gastro-intestinal tract removed. The carcass was ground into 95 per cent alcohol and extracted continuously with alcohol for 8 hours. The extract was saponified by the addition of sufficient potassium hydroxide to give a final 1-M solution followed by refluxing 2 hours. After cooling, the alkaline alcoholic solution, *without the addition of water*, was extracted directly with a *single* portion of petroleum ether. It was found that this procedure quantitatively recovered methylcholanthrene added to a rat carcass Dilution of the alkaline solution with water or repeated extraction of the solution with petroleum ether increased background absorption and is unnecessary, since it was found that a single extraction with petroleum ether removes more than 90 per cent of the methylcholanthrene from a solution in 90 per cent ethanol.

The young were skinned, and gastro-intestinal tracts and skinned carcasses were separately pooled, extracted with alcohol, saponified, and extracted with petroleum ether as described above.

The curves in Figure 2 indicate that methylcholanthrene can indeed be excreted in the milk. The presence of inflections in the absorption curves at 284 and 295 mμ of the extracts of the carcasses and intestinal tracts of the young is taken as definite evidence for the presence therein of the carcinogen. By comparing this curve with those in Figure 1, we can make a rough estimation of the quantity of the carcinogen present, the total amount in carcasses and intestinal tracts of the whole litter being about 50 μg.

Intravenous and intraperitoneal administration.—The absorption curve for the mother's carcass (Fig. 2) showed that only extremely small quantities of methylcholanthrene were being absorbed from the intestine, a finding which is in good agreement with the recent observations of Heidelberger and Jones (3) with radioactive 1,2,5,6-dibenzanthracene. Since transfer of the carcinogen to the offspring by way of the milk was doubtless being limited by its absorption from the gastro-intestinal tract of the mother, it was anticipated that intravenous or intraperitoneal administration of the carcinogen would raise the levels considerably and hence would increase its mammary excretion. This expectation was borne out as shown by the absorption curves in Figure 3. For intravenous administration, an emulsion of the carcinogen was injected into the tail vein. The emulsion was prepared as follows: a solution of 20 mg. of methylcholanthrene in about 3 ml. of acetone was added in small portions, with stirring, to a boiling solution of 50 mg. Emulsone B (a nontoxic emulsifying agent manufactured by the Glyco Products Co.) in 10 ml. of water. The emulsion was then concentrated to 10 ml., yielding a fairly stable nontoxic suspension with a final concentration of 2 mg. methylcholanthrene per milliliter.

A female rat, nursing a litter of seven, was given 1 ml. of the emulsion intravenously on the tenth day *post partum*. Five hours later the mother and litter were sacrificed and separately extracted as described previously. The inflections at 284 and 295 mμ on the absorption curves indicated the

presence of the carcinogen in the bodies of the young rats; the amount was roughly estimated at 40 μg. In similar experiments after intraperitoneal injection of 8 mg. of the carcinogen in 1 ml. of olive oil, it was again demonstrable in the bodies

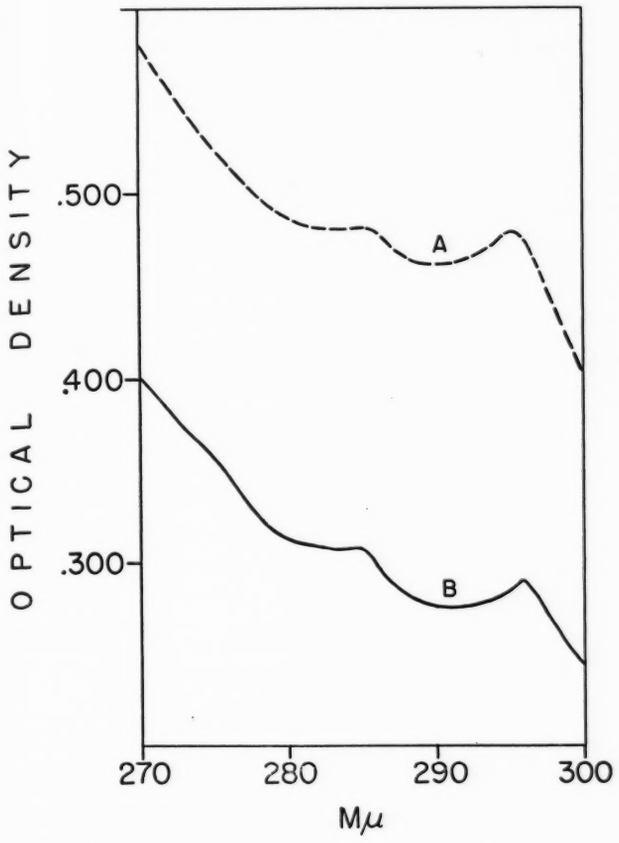

FIG. 3.—Absorption curves of extracts of bodies of young sacrificed 3 hours after administration of methylcholanthrene to the mother by intraperitoneal (curve *A*) and intravenous (curve *B*) injection.

of the young; the amounts present ranged from about 30 μg., in those sacrificed 3 hours after injection of the mother, to approximately 230 μg. in those sacrificed 24 hours after the injection.

In two experiments, one after intravenous, the other after intraperitoneal injection, evidence was obtained for the presence of the carcinogen in the milk. In each experiment, 5 hours after injection of 2 mg. of methylcholanthrene in the mother, the young were sacrificed and the milk removed as a coagulated mass from the stomachs. After extraction and saponification essentially as described for the other tissues, extracts of the unsaponifiable matter of the milk gave the curves shown in Figure 4. The amounts of methylcholanthrene present therein are estimated to be of the order of 20–30 μg.

To test the possibility that the excreted material was a degradation product rather than the intact hydrocarbon, an experiment was performed with radioactive methylcholanthrene. By isolating pure radioactive methylcholanthrene from the milk by the carrier technic, it was possible to establish definitely that this carcinogen can be transferred intact by way of the milk to the offspring. The radioactive 20-methylcholanthrene, labeled with C^{14} in the 11-position, was obtained through the kindness of Dr. W. G. Dauben of the Department of Chemistry of the University of California. It had an activity of 100,000 counts/min/mg, which in our Q gas counter corresponded to a specific activity of 280,000 counts/min/dish.

A rat, 8 days *post partum*, received 5 mg. of the radioactive carcinogen by intraperitoneal injection in 2 ml. of olive oil. After 5 hours, during which time the young nursed freely, the entire litter of eight was sacrificed. Each one was carefully skinned, the stomachs opened, the milk contained

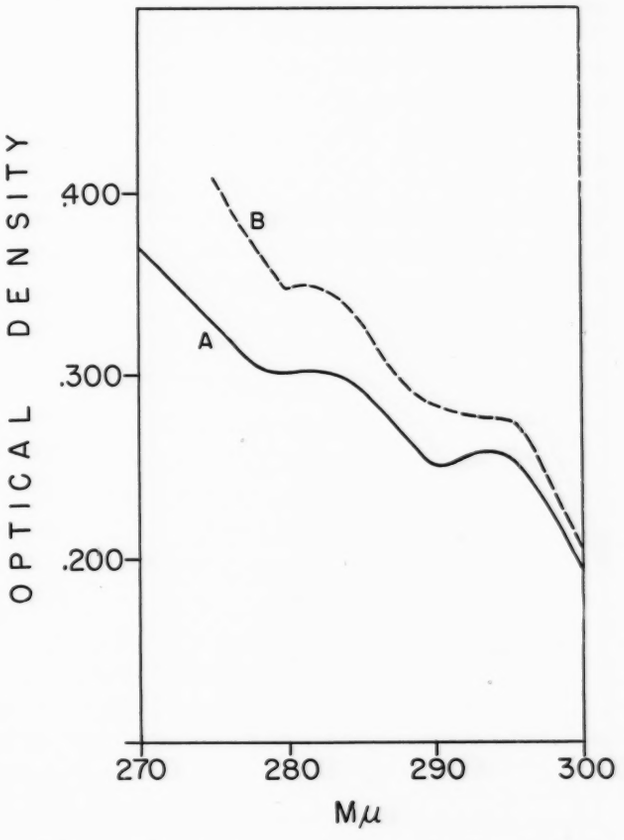

FIG. 4.—Absorption curves of extracts of milk taken from stomachs of young sacrificed 5 hours after administration of methylcholanthrene to the mother by intraperitoneal (curve *A*), and intravenous (curve *B*) injection.

therein pooled, and the unsaponifiable matter extracted as described above. This material, in an amount of 28 mg., gave an absorption curve similar to Figure 4, corresponding to approximately 5 μg. of hydrocarbon. When counted on a 7.5 sq.

cm. dish in a flow counter, it had an activity of 39 counts per minute above background. To this material there was added 10 mg. of nonradioactive carrier methylcholanthrene, and the mixture crystallized from alcohol. The specific activity, corrected for layer thickness, was 63 counts/min/dish. On a second crystallization from alcohol, the activity increased to 71 counts/min; and on the third crystallization from acetone, the activity remained essentially constant at 68 counts/min. By a similar procedure it was also possible to demonstrate the presence of the intact carcinogen in the skinned carcasses of the young. On the basis of the specific activity of the original methylcholanthrene of 280,000 counts/min/dish, we can calculate that the milk contained $68 \times 10{,}000/280{,}000 = 2.5$ μg. of the carcinogen. This value is similar in magnitude to the value of 5 μg. obtained from ultraviolet absorption. This, of course, represents only a minute fraction of the total quantity of 5 mg. administered, but the data evidently leave no further doubt concerning the mammary excretion of methylcholanthrene.

The mammary excretion of a polycyclic hydrocarbon such as methylcholanthrene is not surprising, in view of the generally well recognized excretion of other lipoids by this route. The fact has long been known that when pungent foodstuffs are eaten by a lactating mother, the essential oils which carry the flavors find their way into the milk. The mammary excretion of vitamin D (4), estrogens (5, 6), and a host of drugs such as DDT (7), thiourea and thiouracil (8), and sulfathiazole (9) have been reported, and there is no reason why such lipid-soluble substances as the carcinogenic hydrocarbons should not behave similarly.

The carcinogenic activity of methylcholanthrene when administered in this fashion by way of the mother's milk is now under further investigation.

REFERENCES

1. Shay, H.; Weinhouse, S.; Gruenstein, M.; Marx, H. E.; and Friedmann, B. Development of Malignant Lymphoma in Rats Suckled by Mothers Receiving Methylcholanthrene. Cancer, **3**:891–95, 1950.
2. Berenblum, I.; Holiday, E. R.; and Vope, E. M. Some Physical Methods of Investigating Carcinogenic Hydrocarbons. Brit. M. Bull., **4**:326–31, 1947.
3. Heidelberger, C., and Jones, H. B. The Distribution of Radioactivity in the Mouse Following Administration of Dibenzanthracene Labeled in the 9- and 10-Positions with Carbon 14. Cancer, **1**:252–60, 1948.
4. Embleton, J., and Collings, A. Transference of Vitamin D from the Female Rat to her Young. Nature, **159**:340, 1947.
5. Courrier, R. Recherches sur la mécanisme de la crise génitale du nouveau. Proc. 2d Internat. Cong. Sex Research. London, 1930.
6. Lacassagne, A., and Nyka, W. Folliculin and Pathogenesis of Mammary Adenocarcinoma. Folliculin in Colostrum. Compt. rend. Soc. de biol., **116**:844–45, 1943.
7. Telford, H. S., and Guthrie, J. E. Transmission of the Toxicity of DDT through the Milk of White Rats and Goats. Science, **102**:647, 1945.
8. Chesley, L. C. The Absorption, Apparent Volume of Distribution, and Excretion of Thiourea. J. Clin. Investigation, **23**:856–58, 1944.
9. Rieben, G., and Druey, J. Excretion of Sulfanilamide and Sulfathiazole in Breast Milk and Its Significance for the Infant. Schweiz. med. Wchnschr., **72**:1376–79, 1942.

Announcement

GOOD NEWS

The Editors are pleased to announce a change designed to improve the quality of illustrations. Starting with the January issue all half-tone illustrations (photomicrographs and photographs) will be printed as plates at the end of each article. A high quality egg-shell type of paper will be used for the text to reduce glare and to improve readability. The plates will be heavy and glossy and will provide contributors with the best facilities for the reproduction of half-tones. While this change will be of interest to all readers, it will be especially welcomed by those demanding the highest quality in the reproduction of photomicrographs.

Authors are urged to use unusual care in the preparation of photographs, since better paper and a finer screen will accentuate poor features of bad pictures as well as the finer qualities of good ones. The reproduction of half-tones is an expensive process. Only those photographs that are essential to the presentation and of the highest technical quality will be accepted. Photographs that duplicate one another or that illustrate well known features should not be submitted.

These changes will necessitate certain minor changes in the preparation of manuscripts. Authors are urged to refer to the new "Information for Authors" on the inside of the back cover for these changes.

ACKNOWLEDGMENT

The Editors are indebted to Dr. Erich Hirschberg for the compilation of the index to Volume 10. Dr. Hirschberg, who was formerly on the staff at theMcArdle Memorial Laboratory, has recently accepted a position at the Institute of Cancer Research, College of Physicians and Surgeons, Columbia University.

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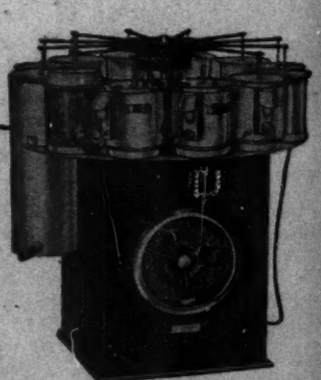

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